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INVASIVE HOLLIES (*ILEX*, AQUIFOLIACEAE) AND THEIR DISPERSERS IN THE PACIFIC NORTHWEST

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ABSTRACT

Naturalized *Ilex aquifolium* L. (English holly) was first collected in the Pacific Northwest in 1953, based on herbarium records. Field surveys showed it is now commonly naturalized from northwestern California to coastal British Columbia. *Ilex crenata* Thunb. and *I. opaca* Aiton were also found growing outside of cultivation, but rarely. A key and seed illustrations are provided to distinguish these three *Ilex* species. Between 2003 and 2006 twice-weekly visits to naturalized and cultivated hollies in Seattle revealed seven species of birds disseminating seeds by eating the fruits. American robins, *Turdus migratorius*, accounted for 96% of 2796 frugivory observations on *I. aquifolium*, followed by European starlings, *Sturnus vulgaris* (3.2%). *Ilex aquifolium* fruits ripened in October and persisted for six months, yet 99% of all fruit was consumed between November and February. A study of *I. aquifolium* seed fate found pre-dispersal diurnal seed predation was rarely observed. Bird-regurgitated seed was more frequently attacked by nocturnal rodents in a sheltered forested setting in Clark Co., Washington (39% losses), compared to an exposed urban setting in Seattle (2% losses). The percentage of viable seed surviving rodent attack was higher in the urban sample (66%) than in the forest sample (24%). Commercial and ornamental use of *I. aquifolium* is extensive in the coastal region and less-invasive alternatives should be considered, to provide food and cover for urban avians without degrading natural areas.

Key Words: American robin, English holly, *Ilex aquifolium*, invasive plants, seed dispersal, seed predators, *Turdus migratorius*.

Holly, the genus *Ilex*, is the largest genus of woody dioecious plants, with more than 500 species worldwide (Cuénoud et al. 2000; Loizeau and Spichiger 2004). More than 30 holly species are cultivated in gardens in western North America, as well as a large number of named hybrids (Omar 1994; Galle 1997; Jacobson 2006). No native *Ilex* species are found on the Pacific coast of North America.

Ilex species are recently escaped (a non-native growing outside of cultivation, without human intervention) or naturalized (a non-native growing and reproducing outside of cultivation) in western North America. *Ilex* ovaries ripen into a drupe, usually containing 3–4 nutlets (pyrenes). For convenience, I refer to these ecological dispersal units (diaspores) as fruits and seeds. Little is known about the interactions between *Ilex* species and their seed dispersers and seed predators of the region, although these data can be important for dealing with invasive species. Therefore, in addition to investigating the collection history and distribution of escaped or naturalized *Ilex* species in the region, preliminary studies on holly dispersal biology are reported here: i.e., feeding behavior of frugivorous birds, and seed fate and viability after bird dispersal.

At least eight English birds, including six thrush species, are known to disperse the seeds of English holly, *Ilex aquifolium* L., in its native range (Snow and Snow 1988). Olmsted (2006) reported some unexpected species consuming holly fruit in the Pacific Northwest, such as (American) blackbirds and chickadees. I attempted to reproduce her findings by systematically observing concentrations of fruiting holly species (naturalized and cultivated) in or near Seattle's Washington Park Arboretum over three years, to resolve which birds were responsible for the most frugivory. In the settled landscape of southern England one study found frequent interactions between avian predators and their prey, flocks of fruit-eating birds, which affected fruit-gathering behavior (Snow and Snow 1986). So I recorded the behavior of urban American robin flocks when gathering fruit.

Seed viability and the fate of seeds handled by birds were examined for possible effects of seed predators in two settings: an urban area and in a typical rural forest. I focused on the most widespread and invasive holly in western North America, *Ilex aquifolium*, and asked what species ate the seeds by day, how frequently, and what percentage of seeds were destroyed by seed predators after they were transported by birds. *Ilex aquifolium* seed is protected by a thick bony exocarp (Fig. 1) and germination is delayed 18–36 mo in Europe (Beckett and Beckett 1979; Arrieta and Suárez 2004). For comparison a three-year outdoor seed germination test was conducted in Seattle.

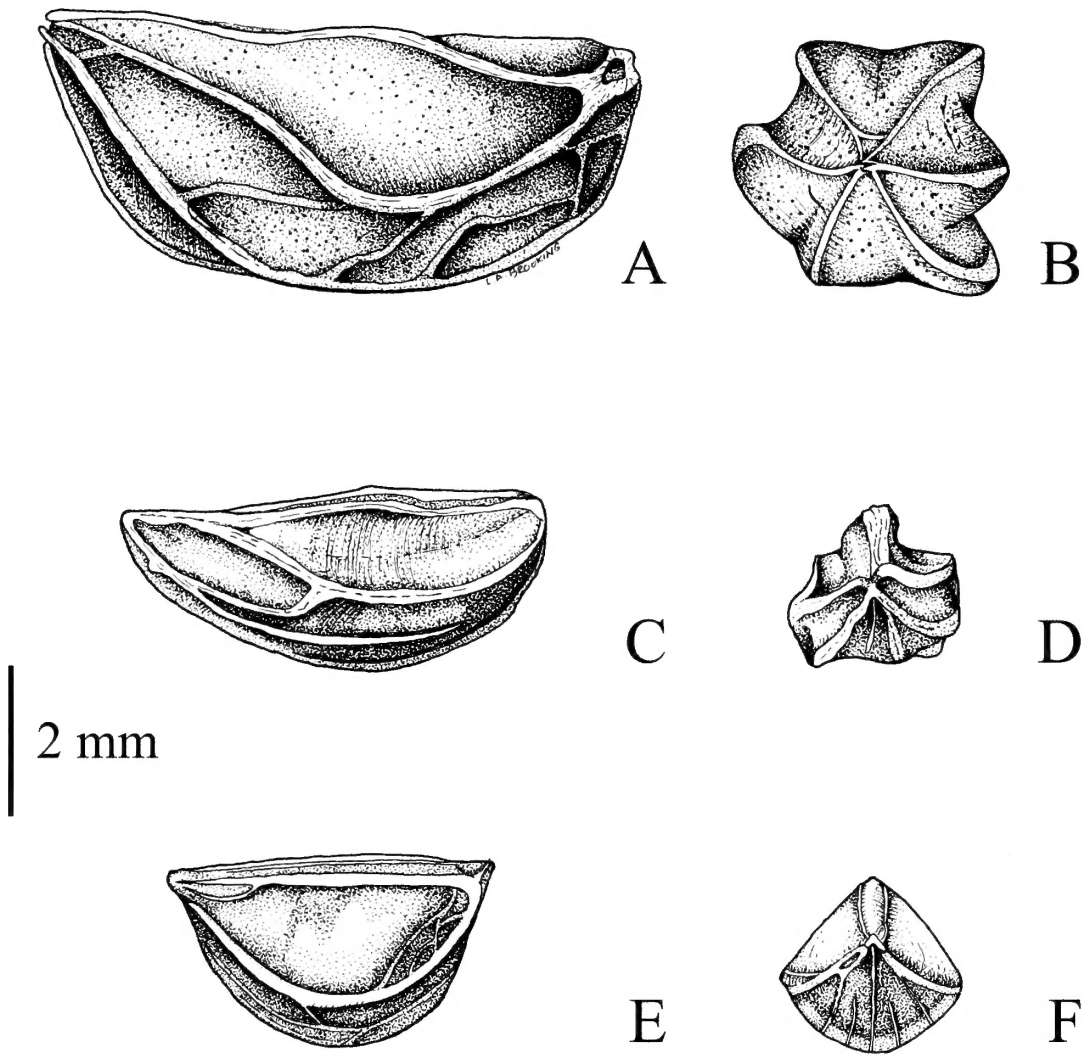

FIG. 1. Seeds of escaped hollies in the Pacific Northwest. *Ilex aquifolium*, (a) lateral view, (b) proximal view. *Ilex opaca*, (c) lateral view, (d) proximal view. *Ilex crenata*, (e) lateral view, (f) proximal view.

METHODS

Distribution

Holly distribution data were compiled from the literature and specimens at the following herbaria: A, BM, CDA, CHSC, COCO, DAV, DAVFP, DBG, DECV, ELRG, FTU, GH, HSC, JEPS, KHD, LINN, MALA, NEBC, NLSN, NY, ORE, OSC, POM, RSA, SCCBC, SFUV, SOC, UBC, UC, UCR, UVIC, V, WILLU, WLK, WS, WTU, and WTUH (acronyms from Holmgren et al. 1990). Additional collections consulted included: the Shasta-Trinity National Forest, Redding, California; Reed College, Portland, Oregon; Olympic National Forest in Olympia, Washington; The Evergreen State College in Olympia; and Fort Clatsop National Memorial, near Astoria, Oregon. The study area was broadly defined as the lowlands west of the Cascade Range in southwest British Columbia, western Washington, and western Oregon. Populations were considered naturalized and mapped if they were obviously not planted and reproducing outside of cultivation, or if herbarium labels indicated they were not cultivated. Field surveys for naturalized holly were conducted on 50 d between 2000 and 2006. Herbarium vouchers from representative naturalized holly populations were deposited at WTU.

Frugivory Studies

The 21 holly taxa in Table 1 were studied at the edge of second-growth forest in the former holly plantings of the Washington Park Arboretum, part of the University of Washington Botanic Gardens in Seattle, King Co., Washington (Omar 1994), or areas within two km of the arboretum, including the University of Washington campus, and the adjacent Montlake neighborhood (Alberti et al. 2001). Frugivory observations were made two times a week during daylight hours between December 2003 and March 2006, while walking to and through the grounds of the arboretum looking for bird activity. All observations of animals eating fruits or seeds were recorded. Individual bird observations began when the first fruit was swallowed and ended when the bird stopped feeding and left the fruit source. It was soon evident that American robins were the most frequent frugivore to visit naturalized *Ilex*, although this aspect of their natural history was not recorded in ornithological literature, so I compiled detailed notes of their feeding behavior. To estimate the transport of fruits and seeds, a count of total English holly fruits swallowed in one feeding bout was made for 25 American robins in Seattle. Ten large robin flocks were also timed (in minutes) when feeding on fruit, starting with the first bird perching on a

TABLE 1. NUMBER OF OBSERVATIONS OF BIRDS SWALLOWING *ILEX* FRUITS IN THE PACIFIC NORTHWEST, 2004–2006. Avians are American robin, European starling (ES), hermit thrush (HT), cedar waxwing (CW), American crow (AC), varied thrush (VT), and northern flicker (NF). Nomenclature follows Andrews (1997).

Ilex	Avian								
	Robin	ES	HT	CW	AC	VT	NF	Total	%
× *altaclerensis* (Loudon) Dallim.	858	87		4				949	19.08
aquifolium L.	2690	90	1	11	2	1	1	2796	56.20
aquifolium × *cornuta*	27							27	0.54
× *attenuata* Ashe	168							168	3.38
× *beanii* Rehder	48		32					80	1.61
ciliospinosa Loes.	20		1					21	0.42
cornuta Lindl. & Paxton	43		1					44	0.88
cornuta × *latifolia* × *pernyi*	95		2					97	1.95
cornuta × *pernyi*	5							5	0.10
crenata Thunb.	40		3					43	0.86
decidua Walter	146			1		1		148	2.98
dipyrena Wall. hybrid	10							10	0.20
integra Thunb.	14							14	0.28
× *koehneana* Loes.	18							18	0.36
latifolia Thunb.	3							3	0.06
maximowicziana Loes.	1							1	0.02
opaca Aiton	338		1	3				342	6.87
pernyi Franch.	37							37	0.74
serrata Thunb.	2							2	0.04
verticillata (L.) A. Gray	163		1					164	3.30
yunnanensis Franch.	6							6	0.12
Total observations	4732	177	42	19	2	2	1	4975	
%	95.12	3.56	0.84	0.38	0.04	0.04	0.02		

fruiting branch and swallowing fruit, ending when the last individual departed. Most frugivory observations were made at close range or with Zeiss 7 × 42 binoculars. Fresh samples of ten fruits were gathered in Seattle and measured for each cultivated species in Table 1 (100 fruits of naturalized *I. aquifolium*), then the seeds were manually extracted, cleaned, counted and measured, to determine the range of fruit and seed sizes and the average number of seeds per fruit.

Seed Predation

Seed predation was detected in several ways. Preliminary study showed birds usually swallowed holly fruits whole and departed, but seed predation was obvious when a bird lingered on the fruiting branch, mashed the fruit in its bill, slowly separating and dropping pulp while extracting, manipulating, and crushing seeds. Squirrels also sat on a fruiting branch, discarding fruit pulp and cracking seeds with their teeth, which was audible from 5 m. Seed predation by birds and squirrels was diurnal, producing small amounts of shredded fruit pulp where they attacked seeds. In contrast, evidence of nocturnal seed predation was indirect. The best evidence came from small gnawed holes in bird-regurgitated holly seeds on the ground, with no adjacent shredded fruit pulp. This was assumed to be (nocturnal) rodents feeding on seed contents; their preference for seeds over fruit flesh shown by untouched freshly fallen fruits within a few cm. Several times in Seattle I saw indications of nocturnal rodents (perhaps a rat sp.) climbing shrubs and feeding on the contents of seeds of *Cotoneaster franchetii* Bois, leaving large amounts of fruit flesh and broken seed husks below the shrub, with many shredded and seedless fruits remaining on the branches. Fruiting hollies were checked for evidence of similar arboreal seed predation by rodents throughout the study, in daylight hours; no direct nocturnal observations of rodents were attempted.

Seed Viability

Seed viability for *Ilex aquifolium* was determined from freshly regurgitated seeds at sites where American robin frugivory was observed along sidewalks and lawn edges in Montlake, Seattle, as well as from second-growth *Pseudotsuga menziesii* (Mirb.) Franco forest near the high school in Camas, Clark Co., Washington. A sample of 500 regurgitated seeds was gathered in Montlake in January 2004, planted in one cm of soil in unirrigated pots left outdoors, and monitored for 3.5 yr to record length of time to germination (Barnea et al. 1991). Additional seeds from the same sites were scored for rodent damage, consisting of a gnawed exocarp and missing embryo. Undamaged seeds were halved with a razor and examined with a dissecting microscope. Grey firm embryos were scored as

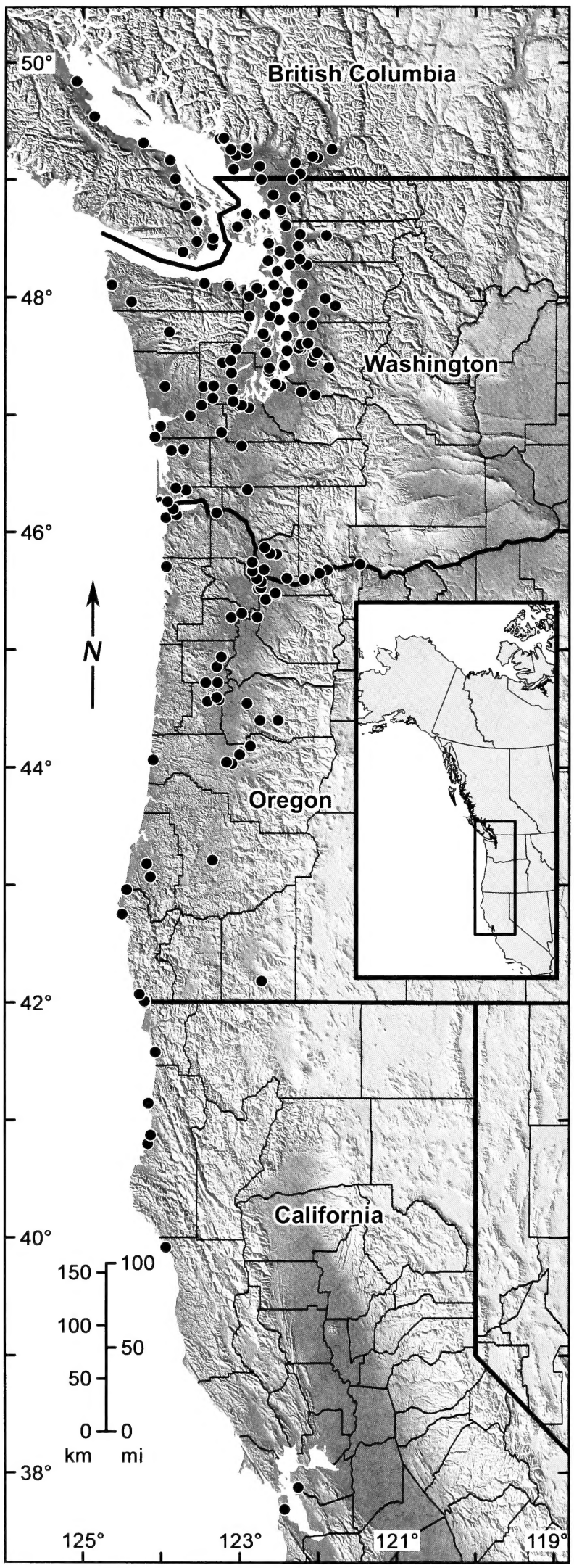

FIG. 2. Distribution map of naturalized *Ilex aquifolium* in western North America, based on herbarium specimens. A few records extend beyond the map boundaries, to the north tip of Vancouver Island in British Columbia (50°35′N, 126°56′W; *Zika 22740* V), and south to Monterey Co., California (36°36′N, 121°54′W; *Zika 23683* RSA).

viable seeds. Liquid, discolored, blackened, or absent embryos were scored as inviable seeds.

RESULTS AND DISCUSSION

Distribution

Literature, herbarium records, and field observations showed three holly taxa escaped from cultivation in northwestern North America: *Ilex aquifolium* (English holly), *I. crenata* Thunb. (Japanese holly), and *I. opaca* Aiton (American holly) (Zika and Jacobson 2005). Their seeds are illustrated in Fig. 1. A key is provided to separate them.

Key to *Ilex* Growing Outside of Cultivation in the Pacific Northwest

1. Leaves less than 30 mm long, less than 15 mm wide, minutely dentate, never spiny; fruit black, 4.8–6.5 mm diam.; seeds nearly smooth . . . . . *I. crenata*
1′ Leaves more than 40 mm long, more than 20 mm wide, entire or spiny-margined; fruit red, 7–13 mm diam.; seeds grooved and strongly ridged
   2. Fresh leaves scarcely shiny or dull above; pistillate flowers solitary and scattered on the twig . . . . . *I. opaca*
   2′ Fresh leaves glossy above, pistillate flowers clustered on short spurs, in subumbels of 1–8 . . . . . *I. aquifolium*

Only *Ilex aquifolium* was abundant enough to represent a conservation concern in the Pacific Northwest; the other hollies were documented as escapes at just one location each. *I. opaca* was vouchered from a single escaped sapling in King Co., Washington (*Zika 20447* WTU). *Ilex crenata* was restricted to two small shrubs on a brushy pondshore in Snohomish Co., Washington (*Zika 20423 & Jacobson* WTU). *Ilex* × *attenuata* Ashe (*I. cassine* L. × *opaca*) was collected as an escape once in 1977 in Sacramento Co., California (Hrusa et al. 2002), but was not recorded escaped in the study area, even though it fruits in cultivation in Seattle.

In the Pacific Northwest I found *Ilex aquifolium* was thoroughly naturalized at low elevations west of the Cascade Range (Fig. 2). I found it reproducing outside of cultivation at hundreds of locations, including forests of all age classes, dominated by *Picea sitchensis* (Bong.) Carrière, *Pseudotsuga menziesii*, *Acer macrophyllum* Pursh, *Alnus rubra* Bong., or *Populus balsamifera* L. subsp. *trichocarpa* (Torr. & A. Gray) Brayshaw. English holly varied from infrequent to common

TABLE 2. MONTHLY FRUGIVORY OBSERVATIONS FOR *ILEX AQUIFOLIUM*, 2004–2006.

Avian	Oct	Nov	Dec	Jan	Feb	Mar	Apr–Sept	Total	%
American robin	7	314	632	872	862	2	1	2690	96.21
European starling		23	51	11	5			90	3.22
Hermit thrush					1			1	0.04
Cedar waxwing					5		6	11	0.39
American crow					2			2	0.07
Varied thrush					1			1	0.04
Northern flicker				1				1	0.04
Total observations	7	337	683	884	876	2	7	2796	
%	0.25	12.05	24.43	31.62	31.33	0.07	0.25		

in fencerows, thickets, roadsides, lakeshores, and floodplains. The majority of naturalized plants were found in rural, suburban, or urban woodlots, fencelines, and hedges, where nearby pistillate cultivated plants provided a seed source. Plant density was highest in some urban greenbelts, with young stands of *Pseudotsuga* and an understory dominated by naturalized *I. aquifolium* rather than native shrubs.

Ilex aquifolium Collection History

English holly was introduced to the Pacific Northwest as an ornamental by 1869 (Ticknor 1986). Fruiting boughs were popular yuletide decorations, so by 1891 the species was established in commercial orchards. A regional industry continues to this day, providing an estimated 85% of the world's crop of cut branches, which totaled 300 tons in 1963 (Ticknor 1986). *Ilex aquifolium* was first noted naturalized in the Pacific Northwest by Brayshaw (1960) and Taylor and MacBryde (1977). Plants were apparently uncommon at first and the species was not included in local and regional floras of the time (e.g., Hitchcock and Cronquist 1961, 1973; Szczawinski and Harrison 1972; Creso 1984). The oldest herbarium collection is dated 1953 (Vancouver Is., *M. C. Melburn s.n.* V). Prior collections, such as a 1931 sheet from the Columbia River Gorge (*Yuncker & Welch 3703* NY) presumably represent cultivated plants as their labels do not specifically state they are escapes. English holly was mentioned as a locally frequent garden escape in British Columbia "on south Vancouver Island, [and] less frequent on the lower mainland" (Douglas et al. 1989). Within a decade it was reported as "frequent in southwestern British Columbia" (Douglas et al. 1998), indicating it was spreading rapidly. In California, *I. aquifolium* was absent from state floras (e.g., Munz and Keck 1965; Munz 1968) until recorded from the northern coast by McClintock (1993). A naturalized plant was first collected in 1976 in Humboldt Co. (*Barker 1594* HSC). In treatments of Oregon plants, Peck (1961) and Thilenius (1968) did not include the species. The first Oregon record was collected in 1986 (*Zika 9818* OSC). More recently Gray (2005) noted *I. aquifolium* was naturalized in both disturbed stands and old growth forests at low elevations west of the Cascade Range. My field surveys disclosed *I. aquifolium* was naturalized in every urban area in western Washington, although the first herbarium gathering was only in 1987 (*Carnevali 203* ELRG). *Ilex aquifolium* was also reported naturalized in Hawai'i (Wagner et al. 1999), New Zealand (Williams and Karl 1996), and Australia (Gleadow and Ashton 1981). Olmsted (2006) reported the species naturalized on the coast of New England, but there are no vouchers at NEBC (R. Angelo, New England Botanical Club herbarium, personal communication), and the report is dismissed here as a mistake for native populations of *I. opa*ca.

Frugivore Studies

The hollies studied (see Table 1) have fruits 5–13 mm diam. and seeds 2–5 mm diam. Apparently none were too large to be swallowed by the local frugivorous birds; seven species were observed swallowing the fruits of the 21 *Ilex* taxa, including cultivated hybrids (Table 1). Native birds were the primary consumers of fruit, but 4% of the feeding observations represent introduced European starlings (*Sturnus vulgaris*). Indigenous birds swallowing *Ilex* fruits, in order of frequency, include: American robin (*Turdus migratorius*), hermit thrush (*Catharus guttatus*), cedar waxwing (*Bombycilla cedorum*), American crow (*Corvus brachyrhynchos*), varied thrush (*Ixoreus naevius*), and northern flicker (*Colaptes auratus*). Robins, often flocking in winter, consumed 95% of the fruit of all combined *Ilex* taxa. Olmsted (2006) reported *I. aquifolium* fruits in the Pacific Northwest provide food for, among others, "...blackbirds, mourning doves, finches, chickadees and non-native house sparrow," but did not provide supporting data, and I was unable to confirm her reports in this study. Those birds were common and seen near or in holly during the three years of field observations, but they ignored *Ilex* fruits.

American robins (Table 2) were responsible for 96% of fruit consumption observations for *Ilex*

aquifolium (n = 2690), and accounted for 99% of the frugivory observed on *I. opaca* (n = 338), and 93% of the frugivory observed on *I. crenata* (n = 40). Robins were common year-round residents in all habitats with naturalized *Ilex* (Sallabanks and James 1999). Published literature documents robins eating the fruits of *I. opaca, I. verticillata* (L.) A. Gray, and *I. decidua* Walter (which are important wildlife food in eastern North America, see Martin et al. 1951), but not the other *Ilex* species in Table 1.

These results suggest that pest control programs for non-native birds like rock pigeons (*Columba livia*) and starlings would have a negligible effect on the dispersal of naturalized holly. On the other hand, the data present a strong argument that urban populations of American robins eat a great deal of *Ilex* in their winter diet, resulting in considerable dispersal of seed into urban thickets and woodlots.

American Robin Feeding Behavior

The local movement of *Ilex aquifolium* seed was easily observed when American robins foraged in the study area between November and February (Table 2), before the onset of spring breeding and a shift in diet to consumption of more invertebrates (Wheelwright 1986). Robins typically foraged in loose flocks of 5–75 birds. Part of the flock advanced towards a fruit tree, in stages, finally arriving and feeding rapidly. Returning to one or several prominent arboreal perches to process the fruit, they maintained a predator watch as a group (Howe 1979; Snow and Snow 1986, 1988; Fleming 1988) before returning to the fruit source. I refer to these lookout points as "relay trees." Flock members repeatedly advanced from the relay tree(s) to feed on fruit. Holly berries were taken while perched, or occasionally snatched in flight. A few fallen fruits were consumed on the ground, or snapped with the bill by leaping from the ground to a low branch. Occasionally fruit was carried away in the bill, and either swallowed or dropped from a new perch.

On *Ilex aquifolium*, feeding bouts for individual American robins in flocks averaged 44 sec, with a range of 10–115 sec. A sample of 100 English holly fruits gave an average of 3.9 seeds per fruit. My observations of 25 robins feeding on *I. aquifolium* gave an average of 5.2 fruits swallowed, or an estimated 20.3 seeds (3.9 seeds/fruit × 5.2 fruits = 20.3 seeds) per feeding bout. *Ilex aquifolium* has relatively large seeds, 5–8 mm. Presumably most were regurgitated within ca. 15 min and very few seeds were defecated (Murray et al. 1993).

Flocks of foraging robins were observed swallowing large numbers of fruits and seeds. In one observation, as many as 157 robins fed undisturbed over a 30 min period on *Ilex aquifolium*, resulting in removal of an estimated 3187 seeds (20.3 seeds/bird × 157 birds = 3187 seeds). For the flock, this observation represents a potential removal rate of 106 seeds/minute (3187 seeds ÷ 30 min = 106 seeds/minute). In another observation, 122 robins fed on *I. aquifolium* over 20 min before scattering at the approach of their major avian predator, a Cooper's hawk (*Accipiter cooperi*). A similar calculation showed they transported an estimated 2476 seeds, removing approximately 124 seeds/min.

American robin flocks commonly used relay trees 10–50 m from the fruit source. Flock members moved holly seeds to many locations, as some birds varied their approach and departure vectors, or fed on more than one fruit species (Kwit et al. 2004). Winter soils were usually unfrozen in the study area, so some flock members occasionally interspersed frugivory with foraging for invertebrates along brushy edges and in lawns, transferring seeds to additional sites. When a predator alarm was given the birds fled, resulting in some robins carrying seeds 500 m before regurgitation. These observations are consistent with those of Holthuijzen and Sharik (1985), who found flock-feeding birds such as American robins, European starlings, and cedar waxwings facilitated long-distance dispersal of large quantities of seed when present. My observations suggest the variable feeding behavior of American robin flocks, with the use of different relay trees, make them effective dispersers for *Ilex aquifolium* (Schupp 1993; Jordano and Schupp 2000).

Seed Germination, Predation and Viability

Germination of *Ilex aquifolium* seed is delayed 18–36 mo in Europe (Beckett and Beckett 1979; Arrieta and Suárez 2004). Regurgitated seeds I gathered and planted January 2004 germinated 29 mo later. Thousands of regurgitated *I. aquifolium* seeds were found under relay trees in Seattle during the study, and it was common to see American robins regurgitate the seeds after feeding on holly fruits. Seedlings were frequent in these sites. I found regurgitated seeds showed no physical differences from seeds extracted from fresh fruits, as did Meyer and Witmer (1998).

Diurnal seed predation of *Ilex* species was rarely observed in the three years of the study, and is apparently insignificant before dispersal. The seeds of *I. yunnanensis* Franch. were taken from fresh fruits once by a spotted towhee (*Pipilo maculatus*). Similarly, introduced eastern gray squirrels (*Sciurus carolinensis*) fed on *Ilex* fruits in Seattle, loudly cracking open the seeds while discarded pulp accumulated below the tree. Squirrels were seen and heard eating the seeds of *I.* × *altaclerensis* (Loudon) Dallim. (n = 15),

FIG. 3. Rodent seed predation of *Ilex aquifolium*, showing gnawed holes and missing embryo, with mm scale.

I. aquifolium (n = 5), *I. cornuta* × *pernyi* (n = 3), *I. decidua* (n = 2), and *I. opaca* (n = 1).

In Spain, Obeso (1998) found evidence nocturnal rodents were climbing trees and taking seeds from fresh *Ilex aquifolium* fruits. I sought similar evidence from nocturnal visitors, such as abundant discarded fruit pulp and gnawed seed cases on the ground directly below numerous slashed and damaged seedless fruits still attached to pedicels on the branches. Diurnal seed predators observed in the study (squirrels and birds) never produced similar displays, they always picked the fruit before removing the seeds. I was able to observe nocturnal rodent damage a few times on fruits of cultivated *Cotoneaster franchetii* in Seattle, but never on holly, although I examined thousands of fruiting holly branches during daylight hours. I did not attempt direct nocturnal observations of rodents interacting with *Ilex* fruits.

In forested settings bird-regurgitated seeds were easiest to find under naturalized pistillate holly trees, as in Europe (Alcántara et al. 2000; Obeso and Fernández-Calvo 2003). Post-dispersal seed predation, evidenced by small gnawed holes and a missing embryo (Fig. 3), was assigned to small nocturnal rodents such as mice (Jones and Wheelwright 1987; García et al. 2005). This type of seed damage differed from diurnal seed predation as practiced by squirrels, which left accumulations of discarded pulp. Squirrels extracted seeds from fresh fruit picked and held in the forepaws, and their damage also differed in that they seemed to crush or crack open *Ilex aquifolium* seeds instead of gnawing small holes in them to remove the embryo. Although they may occasionally do it, I never saw squirrels gather or eat scattered regurgitated holly seeds on the ground. So I scored the damage shown in Fig. 3 as nocturnal, not diurnal, rodent seed predation.

TABLE 3. NUMBER OF VIABLE SEEDS OF *ILEX AQUIFOLIUM* AFTER RODENT PREDATION, IN URBAN (SEATTLE, KING CO.) AND FORESTED (CAMAS, CLARK CO.) SITES, DETERMINED BY SECTIONING REGURGITATED SEED SAMPLES.

Seed type	Forest		Urban	
	N	%	*N*	%
Rodent damage	300	39	43	2
Viable	184	24	1403	66
Inviable	285	37	680	32
Total	769		2126	

My examination of regurgitated *Ilex aquifolium* seeds in woodland settings and edges invariably showed significant nocturnal rodent predation, as noted in Europe (Smal and Fairley 1982; Obeso 1998; Kollmann and Buschor 2002). Nocturnal rodents damaged 39% of regurgitated *I. aquifolium* seed sampled on the ground near pistillate *I. aquifolium* trees in sheltered forest and forest edge settings in Clark Co. (Table 3). These results are qualitatively similar to studies of *I. opaca* in the eastern United States (Kwit et al. 2004) and *I. aquifolium* in Spain (García et al. 2005; Arrieta and Suárez 2005). In contrast, Seattle's urban walks, lawns, and hedges near cultivated *Ilex* trees had a substantial seed rain that was largely ignored by seed predators, with only 2% post-dispersal seed predation by nocturnal rodents. The general lack of cover and suppressed seed predation together suggest a powerful nocturnal predator influence, possibly urban cats (Crooks and Soulé 1999; Haskell et al. 2001).

A secondary effect on seed viability may also result from the differences in post-dispersal seed predation in forested and urban sites. In the forested sample (n = 769), 76% of the regurgitated seed was either damaged by nocturnal rodents or was inviable (Table 3). In the urban sample (n = 2126), only 34% of the seed was either damaged by nocturnal rodents or was inviable. Said differently, 24% of surviving seed was viable in the forest, compared to 66% in the urban sample. Nocturnal rodents may be able to detect and ignore inviable seed in the forest, and apparently are unable or unwilling to attack viable seed in exposed situations in urban settings. Although these are small samples, the

seed studies suggest post-dispersal seed predation is negligible for bird-disseminated holly seed in cities, and may provide a partial explanation for the relative success of *I. aquifolium* in urban and residential areas (Kollmann 2000).

Conservation and Horticultural Implications

Invasive woody plants in North America raise numerous conservation concerns, altering plant communities and displacing the native biota (Catling 1997; Pimental et al. 2000; Friedman et al. 2005; Reinhart et al. 2006). *Ilex aquifolium* is dispersed by the ubiquitous American robin and colonizes forests, edges, and settlements. It represents a long-term management problem in natural areas (Mack et al. 2000; Reichard and White 2001; Dlugosch 2005). As Temple (1990) and Low (2002) discussed, attempts to control or restrict sale of invasive but popular ornamentals like *I. aquifolium* are not always welcomed by gardeners or distributors. Improved public outreach and education are needed, as are ecologically benign substitutes. Table 1 suggests winter-fruiting *Ilex* alternatives exist in the garden trade. They are attractive ornamentals offering cover and food for winter bird flocks, but are apparently non-invasive, as measured by the lack of herbarium records of plants collected outside of cultivation, and an absence of seedlings around irrigated pistillate plants in gardens or arboreta. These all are in contrast to *I. aquifolium*, with many adventive herbarium vouchers and which produces numerous seedlings in the immediate area of pistillate plants. However, potential hybrid replacements for *I. aquifolium* in Table 1 should be tested for seed viability (perhaps a proxy for invasiveness), and monitored for their capacity to reseed in our climate over a longer period than this study. Nonetheless, some hollies seem to show promise as horticultural options preferable to *I. aquifolium*. These include red-fruited deciduous shrubby species like *I. decidua* and *I. verticillata*, popular with birds in Seattle and in England (Ridley 1930). *Ilex* × *meserveae* S. Y. Hu is a little-known low-growing *I. aquifolium* hybrid (*I. aquifolium* × *rugosa* F. Schmidt) but its fruits were outnumbered and ignored by birds in the study area. Landscapers might instead favor evergreen trees with the form as well as the color of English holly, like *I.* × *koehneana* Loes. (*I. aquifolium* × *latifolia* Thunb.), *I.* × *beanii* Rehder (*I. aquifolium* × *dipyrena* Wall.), and especially *I.* × *altaclerensis* (*I. aquifolium* × *perado* Aiton). The latter accounted for 19% of all *Ilex* frugivory observations, and has the dense growth, bright fruit color, and dark shiny foliage most similar to *I. aquifolium*. From an ornamental, ornithological, and invasive standpoint, *I.* × *altaclerensis* may be the best available replacement, based on my initial results. However, any holly must be rigorously tested for invasiveness, and should be commercially available, before promotion as an alternative to *I. aquifolium* in the Pacific Northwest.

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DISTURBANCE, RESOURCES, AND EXOTIC PLANT INVASION: GAP SIZE EFFECTS IN A REDWOOD FOREST

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ABSTRACT

Fluctuations in plant resource availability are hypothesized to promote exotic plant invasion by allowing propagules already present in an area a chance to successfully compete for unused resources. To examine the relationship between resource enrichment and exotic species invasion, we used selective logging canopy gaps over a range of sizes (56 m^2 to >1500 m^2) in a redwood forest (Santa Cruz County, CA) as a surrogate for disturbance intensity and level of pulsed resource enrichment. Measurements of abiotic conditions in gaps ca. 10 yr after logging suggest light is the primary difference in current resource availability, though a pulse of light and nutrients likely occurred at the time of gap formation. Exotic species richness and relative cover increased significantly as gap size increased. In a separate manipulative experiment, we compared understory plant composition between artificially shaded and unshaded plots in 2.5-year-old logging gaps. Shaded plots had a lower proportion of exotic species than did adjacent, unshaded plots, showing that light is a critical resource for exotic species in redwood forest habitats. Taken together, these results support the view that both physical disturbance and increased availability of scarce resources contribute to a community's susceptibility to invasion and suggest a linear relationship between the size of logging gaps and the magnitude of exotic species invasion.

Key Words: Canopy gap, disturbance, redwood forest, selective logging, *Sequoia sempervirens*, understory.

The probability of invasion by non-native plant species is determined by the supply of introduced propagules, the capacity of these species to establish, and the susceptibility of the environment to invasion (Lonsdale 1999). Susceptibility, or invasibility, of the environment is determined by bottom-up forces (such as light and nutrients), top-down forces (such as herbivores and pathogens) and lateral forces (facilitative and competitive interactions among plants) (Davis et al. 2000). Theories on invasibility often focus on the dynamics of bottom-up forces and suggest that increases in resource availability (e.g., light, moisture, nutrients) promote invasibility of plant communities. For example, increased water supply in drought-prone areas often promotes invasion (Li and Wilson 1998; Davis et al. 1999; Dukes and Mooney 1999) as does the addition of limiting nutrients in North American grasslands (Stohlgren et al. 1999). Alternative theory suggests that physical disturbance acts by disrupting existing species interactions, diminishing the competitive intensity for resources within plant communities, and thus allows foreign invaders to take a foothold (Rejmanek 1989; Hobbs and Huenneke 1992). Disturbance may also increase unused resources in a community by disrupting resource uptake. Davis et al. (2000) suggest that it is the presence of unused resources rather than total amount of resources that is critical to invasive species success.

While studies confirm that both physical disturbance and changes in resource availability promote exotic species invasion (Li and Wilson 1998; Stohlgren et al. 1999; Rodgers and Parker 2003; Glasgow and Matlack 2007), the magnitude of change attributable to each of these factors is rarely studied. However, we know that plant competitive intensity declines as the magnitude of unused resources increases (Davis et al. 1998). Therefore, a large increase in resource availability should boost the success of exotic species invasions.

Canopy gaps caused either by natural treefalls or logging events are one type of disturbance that increases local resource availability. Canopy gap formation causes an immediate resource pulse at the forest floor. The quantity and quality of light increase proportionate to the amount the overstory shade is diminished (Collins et al. 1985). Higher precipitation throughfall and lower transpiration may cause soil moisture to increase (Collins et al. 1985), but this trend may be mediated in areas with coastal fog (Dawson

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1998). Soil disturbance caused by fallen or extracted trees can also create a mineral soil seedbed critical for plant germination (Battles et al. 2001). Decaying plant debris from fallen or extracted trees, in combination with reduced plant uptake, increase nutrient availability (Matson and Vitousek 1981; Vitousek 1985a, b; Frazer et al. 1990). Eventually these abiotic resources return to base levels, but return time varies among these resource classes and could be quite long in areas where soil mineralization rates are low. Surplus nutrients that are not absorbed by rapidly colonizing plants are lost, in the relatively short term, through erosion and leaching (Uhl et al. 1982; Vitousek 1985b). Similarly, excess soil moisture and newly formed mineral soil seedbeds will decline as plants and their roots re-colonize empty space above and belowground. However, light levels decline slowly and canopy closure may take years or decades to complete (Moore and Vankat 1986). Thus, canopy gaps cause a resource pulse whose components re-equilibrate at different rates.

Canopy gaps cause measurable changes in herbaceous species composition in forest ecosystems (Davison and Forman 1982; Moore and Vankat 1986; Glasgow and Matlack 2007). Moore and Vankat (1986), for example, found that while total species richness remained unchanged in canopy gaps, species composition changed substantially with early spring annuals declining and late spring and summer species becoming more abundant. California's coastal redwood forest communities tend to be composed of native species, with low light levels in the understory providing a potential barrier to colonization by the many exotic plants that thrive in disturbed sites in the region. Selective logging events are different than natural treefalls as they remove large merchantable tree boles while unmerchantable stumps, branches and leaf litter remain. Experiments using selective tree removal have found that changes in understory plant composition are similar to those in natural treefall gaps of similar size (Collins et al. 1985; Collins and Pickett 1988a).

We conducted two complementary field studies using canopy gaps formed during selective logging to examine the effects of physical disturbance (tree removal) and resource pulses on exotic species invasion in a coast redwood (*Sequoia sempervirens* (D.Don) Endl.) forest. In the first study we used forest canopy gaps of different sizes that were created in the 1990's by selective logging operations to examine the effects of logging disturbance magnitude on invasibility in the understory plant community (referred to as the *gap size* study). Gap sizes in this study encompassed a range of over an order of magnitude in area (56 m^2 to 1612 m^2) and were of similar size to natural treefalls found in redwood forests (160 m^2 to 1770 m^2) (Sugihara 1992; Busing and Fugimori 2002) as well as other temperate forests (8 to 1320 m^2) (Barden 1981; Romme and Martin 1982; Collins and Pickett 1988b). In the second study we tested for a direct effect of light as a pulsed resource after logging by using paired, artificially shaded (using shade cloth) and unshaded plots in newly created logging gaps in the same forest (referred to as the *light effect* experiment).

In these studies, we tested the hypothesis that exotic plant species survival and dominance are positively influenced by physical disturbance (tree removal) and resource pulses (light and nutrients) created by gap formation during selective logging. We predicted that exotic species richness and cover would increase concomitantly with the size of canopy gap, in the gap size study. In the light effect experiment, we expected that sections of canopy gaps covered by shade cloth would experience a reduced influx of exotic species after gap formation when compared to unshaded regions of the same canopy opening.

Methods

Study Site

For both experiments, we used selective logging sites in a redwood forest located in the Santa Cruz Mountains at Swanton Pacific Ranch, ca. 21 km north of Santa Cruz, CA (37°04′N. 122°14′W), a 3200 acre property owned and managed by the California Polytechnic University, San Luis Obispo. The region receives approximately 700 mm of rainfall annually, mostly between November and May, and has a mean temperature of 13C. The forest, which was clear-cut in the early 1900's, is dominated by coast redwood (*Sequoia sempervirens*) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). In 1991 and 1995, California Polytechnic designated forest sections to be selectively logged. They removed individual and small stands of trees, leaving canopy gaps of various sizes scattered within the forest. For the gap size comparison, we established 16 understory plant census plots (8 × 8 m) in clearings under canopy gaps. All gaps were located in an area of ca. 0.5 km^2 (Fig. 1). Gaps were identified through a comprehensive search of the logged area and all gaps over 100 m^2 were used. The shade cloth experiment was established in two sections of the same forest, after a third selective logging operation completed in November 2004. These sections were not affected by the 1991 or 1995 logging events. For this second experiment, we established paired plots (shaded and unshaded) in 14 clearings under logging gaps.

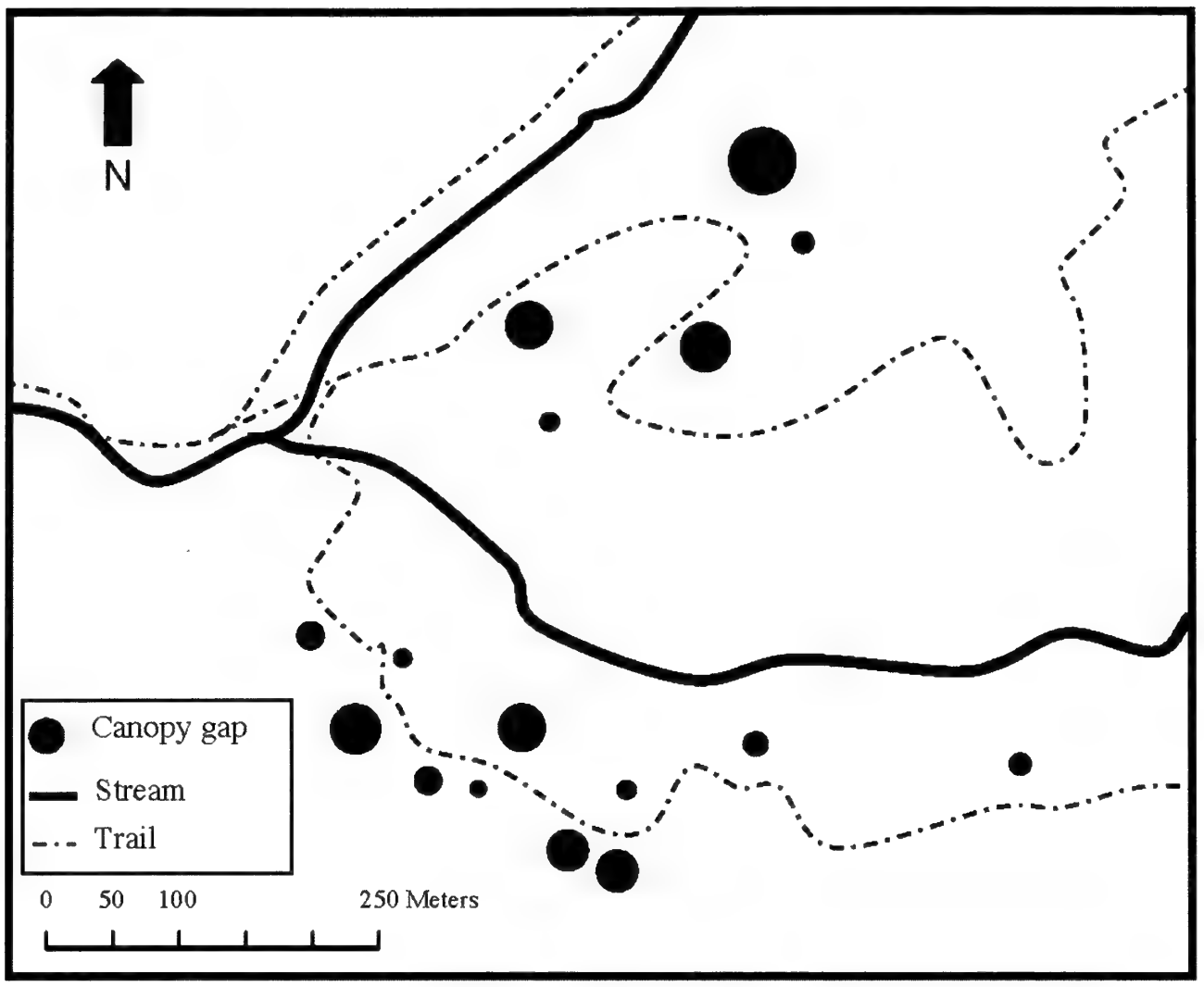

FIG. 1. The location of canopy gaps at Swanton Pacific Ranch (n = 16) used in gap size comparison are represented by black circles showing relative sizes and proximities.

Gap Size Effects

Plot evaluation. For the gap size comparison, gaps were initially identified as clearings of >100 m^2 with no mature tree stems. Canopy gap openings were measured in each of eight compass directions as the distance from plot center to points directly below the edge of surrounding canopy foliage (Brokaw 1982). Hemispherical photographs were taken with a Nikon 6006 camera (Nikon, Melville, NY, USA) and a Peleng 8-mm fisheye lens (Peleng, Minsk, Belarus) using Kodak Elite-Chrome film (200 ASA, Eastman Kodak, Rochester, NY, USA). The camera was mounted on a tripod, pointed skyward, and positioned so that the top of the photograph corresponded to due north. After leveling the camera, two photographs were taken at each plot. The photograph at each site with the best contrast was used for analysis. Slides were digitized using a Polaroid Sprint 35 mm scanner (Boston, MA, USA). Images were then analyzed using the computer program Gap Light Analyzer 2.0 (Frazer et al. 1999) to determine percent transmitted global photosynthetically active radiation (PAR). Percent transmitted PAR represents the amount of above-canopy direct and diffuse PAR incident beneath the canopy (Canham 1988).

To determine temperature and humidity we placed data loggers and probes (Campbell, Logan, UT, USA) at the center of six plots that represented the range of gap sizes present in this study. We measured temperature and relative humidity in five plots and temperature in the sixth between July 18 and September 17, 2003. This period represents the warmest and driest weeks of the year (Mediterranean climate) when temperature and humidity differences between small and large gaps should be most pronounced. Data loggers calculated minimum and maximum daily temperature and relative humidity. We conducted linear regression analysis (SPSS 1999) to determine the effects of gap size on temperature and humidity.

Soil properties. To determine if soil moisture (surface and root zone) varied with gap size in the wet and dry season we measured water content in each plot, using two 5 cm-diameter soil cores from a 0–5 cm and 5–20 cm depth in June (dry season) and November (wet season), 2003. Fresh 10 g sub-samples from each core (4 per plot) were oven dried at 90C to constant weight and subsequently weighed to calculate water content. The levels of essential plant nutrients (N, P, K, Mg and Ca) in the plots were determined from the remaining soil in the 0–5 cm cores. These

samples were air dried and passed through a 2 mm sieve. The two dried 0–5 cm soil samples from each plot were pooled and mixed thoroughly before analysis for available N (nitrate and ammonium), P, K, Mg and Ca. Soil N, Mg and Ca were determined using soil sub-samples extracted with a sodium acetate solution and P was extracted with Bray's solution (Page et al. 1982). Soil chemical analyses were carried out at Perry Laboratories (Watsonville, CA, USA). Gap size effects on soil properties were examined using regression analysis (SPSS 1999).

Vegetation purveys. We surveyed each of the sixteen 8 m × 8 m plots in April of 2003, during the peak flowering season for understory herbs (February–May), to determine the total number of plant species and the number of exotic plant species present. We estimated plant cover for each species in the center 6 m × 6 m area of each plot, to reduce potential edge effects. Within this area, we randomly chose nine of the possible thirty-six 1 m^2 subplots. For each of the 9 subplots, a 50 cm × 50 cm grid with twenty-five 10 cm × 10 cm cells was held over the vegetation at approximately 1 m height. The number of cells (0–25) that contained a particular species served as a measure of relative cover for that species.

We quantified plant species richness and diversity by using the number of species per unit area in the whole plot censuses (S_t), and a measure of evenness (J). The index J is defined as follows:

$$J = \frac{-\sum_{i=1}^{S_t} P_i \ln P_i}{\ln S_t};$$

where P_i is the relative frequency of occurrence of every species in each plot's nine point-count subplots, and S_t is the total number of species in each 64 m^2 plot.

Exotic (S_e) and native (S_n) species richness were determined using the whole plot (8 m × 8 m) census data. To determine exotic species relative cover (C_e) we used the quadrat cell count data to obtain percentages for each plot. The index C_e is defined as follows,

$$C_e = \frac{\sum_{i=1}^{S_e} e_i}{\sum_{i=1}^{S_e} e_i + \sum_{i=1}^{S_n} n_i};$$

where e_i is the number of occurrences of exotic species i in the nine 25 cell grids, n_i is the number of occurrences of native species i in the nine 25 cell grids, S_e and S_n are the total number of exotic and native species found in the plot. To evaluate treatment effects on species diversity and composition we conducted regression analyses (SPSS 1999). The effects of gap size and percent canopy cover were tested on the number of exotic (S_e) and native (S_n) species, exotic relative cover (C_e), and evenness (J). The index C_e was square-root transformed prior to analysis to meet assumptions of normality.

Light Effects – Experimental Manipulations and Vegetation Survey

In April of 2004, 12 clearings (>100 m^2) under closed canopy were identified by their proximity to trees to be removed during the upcoming logging operation. We surveyed each of the clearings to determine the total number of native and exotic plant species present. After tree extraction in October of 2004, two additional clearings were added and one of the previous sites was discarded due to lack of increased light penetration at the forest floor after logging. The remaining 11 previously surveyed and 2 new clearings corresponded to areas of increased light penetration (canopy openings). Within each of the 13 clearings, we demarcated a pair of 5 m × 5 m plots, and randomly assigned the shade treatment to one plot in each pair. The remaining plot was used as an open (unshaded) control. In January 2005, shade cloth (80% shade) was suspended from a PVC frame 1.5 m in height, with a 2 m T-bar in the center to elevate the center of the shade canopy and reduce litter accumulation on the structure. Shade cloth was draped over the edges of the structure but left 1 m above the ground uncovered to allow access by arthropods and ensure airflow. Each pair of plots (shaded and open) was established within 10 m of each other within a single clearing. When on a slope, paired plots were positioned to have the same slope aspect. In July 2007, all pairs of shaded and open plots were censused for the total number of native and exotic plant species. The relative number of plant species in shade and open plots was compared using a paired t-test for equal variances on the normally distributed differences between light and dark plots for total plant species, native plant species, and exotic plant species per plot (SPSS 1999).

Results

Gap Size Effects

Abiotic factors. Percent transmitted global PAR increased significantly with gap size (Table 1). Average maximum daily temperatures also increased with gap size (Table 1). However, minimum daily temperatures were similar across all gap sizes. In smaller gaps, we measured higher minimum humidity levels than in larger gaps, but maximum humidity levels were similar (Table 1).

TABLE 1. EFFECT OF CANOPY GAP SIZE ON GAP PROPERTIES. Significant P values are indicated in bold.

Property	Coefficient	R^2	F (N)	P-value
Transmitted global PAR (%)	0.010	0.61	14.91 (16)	**0.002**
Native species richness	0.004	0.24	4.43 (16)	0.054
Exotic species richness	0.002	0.71	33.68 (16)	**<0.001**
Exotic relative cover	0.0002	0.41	9.91 (16)	**0.007**
Temperature (C°)				
Minimum	−0.002	0.32	1.89 (6)	0.241
Maximum	0.006	0.83	19.40 (6)	**0.012**
Humidity (%)				
Minimum	−0.040	0.82	13.93 (5)	**0.034**
Maximum	−0.003	0.54	3.47 (5)	0.159

Soil moisture was significantly greater during the wet season than the dry season ($t = 8.11$, $df = 15$, $P < 0.001$). However, sampling within each period showed no significant differences in moisture levels between gap sizes at the 0–5 cm or 5–20 cm depths. No gap size-dependent trends were found for nutrient availability.

Vegetation. The understory vegetation in selectively logged redwood forest gaps was typical of that found in natural forest. Common native species such as *Oxalis oregana* Nutt. (redwood sorrel), *Polystichum munitum* (Kaulf.) Presl. (western sword fern), *Rubus ursinus* Cham. & Schlecht (California blackberry), *Stachys bullata* Benth. (California hedge nettle), and *Trillium ovatum* Pursh. (western wake-robin) were present. The five largest canopy gaps (832 m^2–1612 m^2) contained between 18 to 27 understory plant species, medium gaps (212 m^2–624 m^2) had 15 to 26 species, and the smallest gaps (<150 m^2) had between 11 and 21 species. The total number of native understory plant species in canopy gaps showed no significant relationship with gap size (Table 1) or light availability, though native species richness tended to increase with gap size (coefficient = 0.004, $r^2 = 0.24$, $P = 0.06$).

In contrast, the number and percent cover of exotic plant species increased significantly with gap size and light availability (Table 1, Fig. 2). The exotic plant species found in understory plots were: *Cirsium vulgare* (Savi) Ten. (bull thistle), *Cortaderia jubata* (Lem.) Stapf (jubata grass), *Erechtites minima* (Poir.) DC. (Australian fireweed), *Myosotis latifolia* Poir. (forget-me-not), *Rubus discolor* Weihe & Ness (Himalayan blackberry), *Vulpia* sp. (annual fescue), and *Torilis* sp. (hedge-parsley). The relationship between gap size and estimated light availability was strong ($r^2 = 0.61$) and both were good predictors for exotic species number ($r^2 = 0.71$ (gap size) vs. $r^2 = 0.82$ (light availability)). However, light availability was a far better predictor of exotic species relative cover ($r^2 = 0.61$) than gap size ($r^2 = 0.41$) (Table 1, Fig. 2). The exotic and native species evenness was unrelated to either gap size (coefficient = 0.0001, $r^2 = 0.15$, $P = 0.134$) or to light availability (coefficient = 0.005, $r^2 = 0.14$, $P = 0.147$).

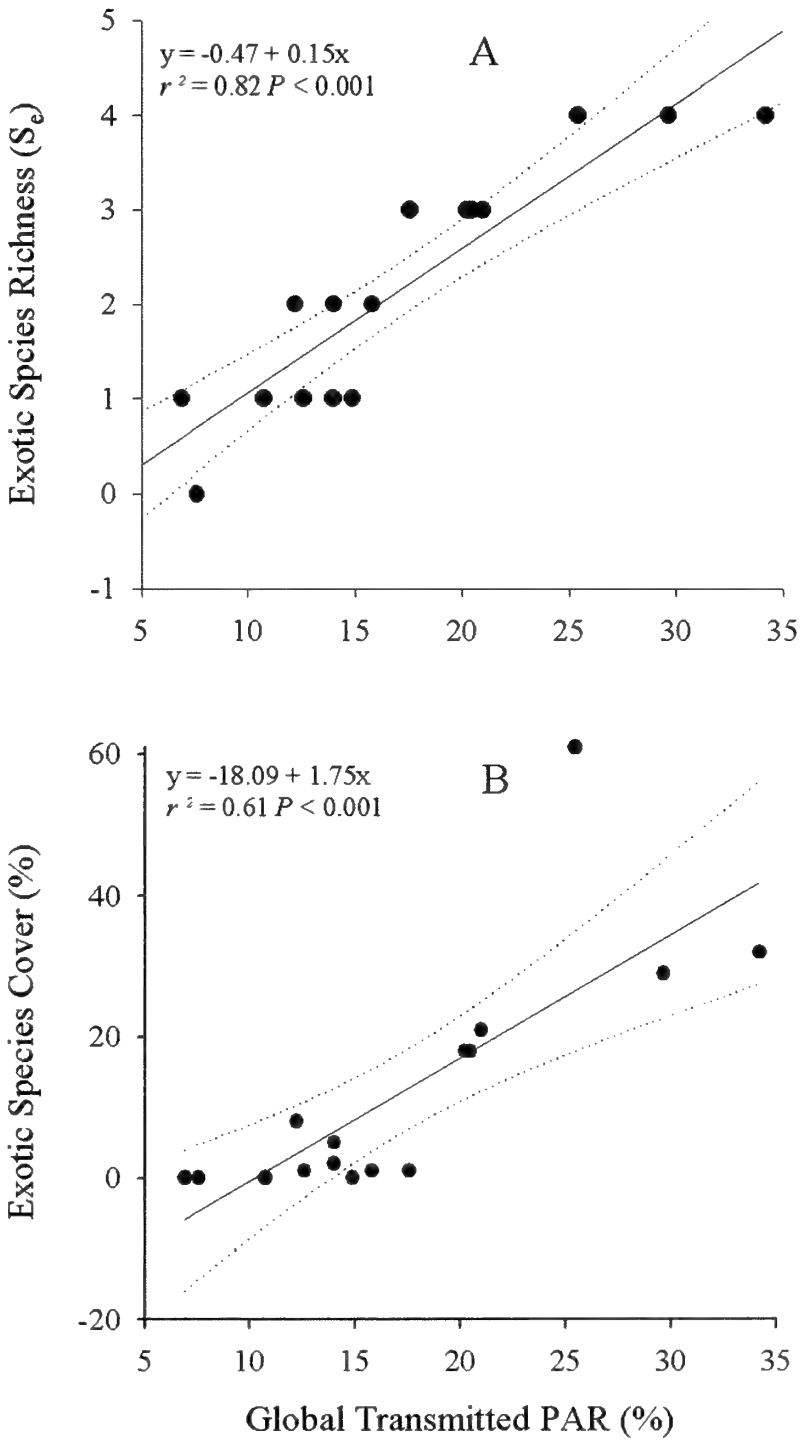

FIG. 2. Relationship between exotic species richness (A), and exotic species relative cover (B) with global transmitted PAR (%). Dotted lines represent 95% confidence intervals.

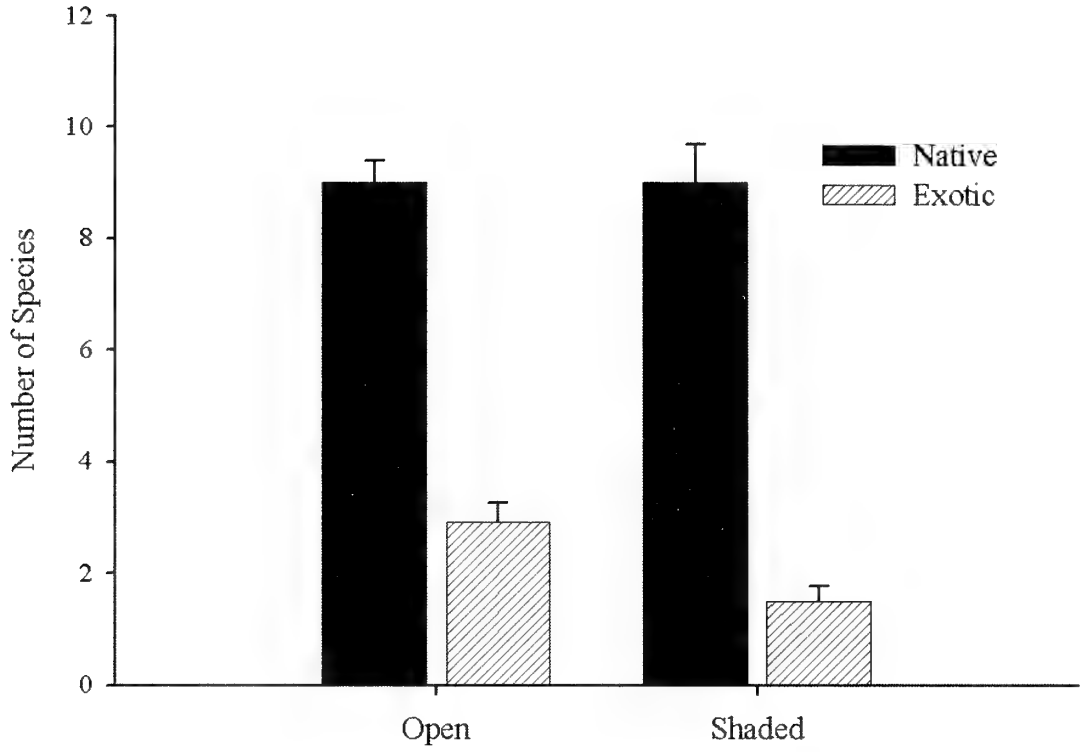

FIG. 3. Mean native and exotic species richness (±SE) in gaps 32 mo after selective logging in artificially shaded and open plots (n = 14 pairs).

Light Effects on Vegetation

The number of plant species occurring in pre-harvest understory in April, 2004 was half the plant species richness occurring within the forest gaps comparison (above) surveyed the previous year (average of 10.3 species in closed canopy before logging versus 20 species, on average, in all gaps). Plant species richness did not increase strongly after 2.5 yr, with a mean richness of 11.9 (±0.5 SE) per 5 m × 5 m light plot (n = 14). However, in the pre-harvest samples, all the species were natives, compared to a mean of 2.9 (±0.3 SE) exotic species per 5 m × 5 m in post-logging open plots. Neither did the addition of shade cloth after logging have a significant effect on plant understory species richness or the number of native species present, on average, in the plot. However, there was twice the number of exotic species present in open plots than in shaded plots ($t = -4.2$, $df = 13$, $P = 0.001$) (Fig. 3).

DISCUSSION

The increasing richness and cover of exotic plant species across selectively logged forest gaps of increasing size supports our prediction that the magnitude of disturbance positively affects exotic species invasion, directly and/or through a resulting pulse in available plant resources. The removal of individuals or clusters of timber trees resulted in direct disturbance of existing vegetation. After disturbance, the amount of light reaching the understory immediately increased and soil likely had a short-term nutrient enrichment as nutrients were released from decaying vegetation and fewer plant roots were present for nutrient uptake (Matson and Vitousek 1981; Vitousek 1985a; Frazer et al. 1990; Frazer et al. 1999). The coupling of decreased resource use by native species with increased total resource availability during the initial disturbance period would have made more resources available to exotic species, which were otherwise suppressed by understory conditions. Over a decade after logging events took place, light availability still varies significantly and directly with the size of the gap created. Invasion of these logging gaps by exotic species and the increase in exotic species richness in gaps of increasing size was likely due to this increase in light availability, and possibly other plant resources whose initial increases are no longer detectable. The persistence of these exotic species is enabled by the length of time required for canopy gaps to close, returning PAR to pre-disturbance levels.

Our shade-cloth study suggests that disturbance and light play complementary roles. There was a doubling of the exotic species richness in unshaded plots after logging compared to the number of species found in shaded plots within the same logging gaps. Further, we found an increase in exotic species even within the experimentally shaded plots compared to the undetectable level of exotic species in our pre-harvest vegetation surveys. Previous studies have found that physical disturbance has the greatest impact on a site's invasibility if it is coupled with increased resource availability (Burke and Grime 1996; White et al. 1997; Leishman and Thomson 2005). Burke and Grime (1996), for example, showed in a manipulative field experiment that, while both physical disturbance and fertilization increased the invasibility of limestone grassland, exotic species were most successful in displacing

their native counterparts when both disturbance and fertilization were present.

However, the conditions under which the exotic species in this study typically occur, and the habits of invasive exotic plants more generally, indicate that light availability may be relatively more important than the influence of disturbance itself, through the physical disruption of established vegetation. Exotic plants, whether intentionally introduced for agricultural or ornamental use (diCastri 1989) or unintentionally introduced (i.e., agricultural weeds) (Heywood 1989), tend to originate from high-light environments. It is thus not surprising that exotics tend to be light-demanding species (Fine 2002) that are shade intolerant (Mack 1996). These characteristics suggest that many exotic species may be successful invaders only after disturbances that increase light availability. Supporting this idea, research in western Oregon found greater numbers of exotic species in the understory of old-growth Douglas-fir forests than in un-thinned second growth forests with lower light availability at the forest floor (Bailey et al. 1998). Exotic species such as *Cirsium vulgare*, *Erechtites minima*, and *Rubus discolor*, found in our study, are shade intolerant and, when found in undisturbed forests, are gradually out-competed by shade tolerant understory plants (Amor 1974; Muldavin et al. 1981; McDonald and Tappeiner 1986).

The importance of treefall gaps in forest ecology is well known. However the impacts of tree harvest on plant community structure and diversity are not clearly understood in Mediterranean forests. In late successional forests, selective logging is often one of the preferred forest management methods because it more closely emulates natural disturbance patterns in uneven-aged forests and maintains mature forest structure (Webster and Lorimer 2002). Studies find selective logging to be superior to other more disruptive management systems (e.g., clearcut and shelterwood logging) in minimizing exotic species colonization (Battles et al. 2001). Unfortunately, several studies suggest that selective logging is disruptive in subtle and indirect ways. For instance, regeneration of certain species is greater with natural gaps rather than logging gaps (Nagaike et al. 1999). Other critics cite the obvious problems and damage that occurs through the tree extraction process (Vasiliauskas 2001) and creation of logging roads and trails (Kreutzweiser and Capell 2001). Old logging roads and trails at our site may serve as pathways for propagules of exotic species into and through the redwood forest habitat (Costa and Magnusson 2002), which can then establish when even low-impact logging techniques are applied.

Perhaps a more pertinent framework is to consider how the forest as a whole responds to artificial gaps in the long term. Even a decade after gap formation, we found obvious vegetative differences among gaps of different sizes. Logging-induced changes in understory species composition are sometimes long-lived (Duffy and Meier 1992; Meier et al. 1995) and forests often revert slowly to their original structure over the course of decades or longer (Alaback and Herman 1988; Halpern and Spies 1995; He and Barclay 2000). In an unlogged forest, succession between periods of natural disturbances (wind, fire) would bring the forest back to its original vegetative composition as shade-tolerant species gradually outcompete the light demanding ones that came in after disturbance. However, because exotic propagules are likely ubiquitous in remaining redwood forests, logging gaps may well be more likely to experience long-term shifts towards exotic composition than natural, pre-invasion treefall gaps.

Exotic plant species are a ubiquitous component of terrestrial ecosystems today, and one that often negatively influences natural habitats (Vitousek et al. 1997). A range of impacts has been documented to occur in terrestrial systems (see Levine et al. 2003 for review). The impact of exotic species in the redwood forest understory is unknown, but their spread in logging gaps may change hydrology, mycorrhizal composition, and interrupt regeneration of disturbance-dependent native species, possibly leading to their extinction. Although exotic species will likely decline locally as gap closure occurs, at a larger spatial scale exotic species are a permanent component of this forest ecosystem. Exotic plants can be expected to take advantage of logging-induced canopy gaps within the forest, highlighting the importance of research on how exotic species impact forest community function. The next step, should their effects be detrimental, would be to explore ways to reduce exotic species spread. Washing logging equipment to limit the spread of exotic seeds, for example, may be a cost effective method to reduce propagule pressure during logging operations (Brooks 2007). While it is inevitable that some form of logging occurs in most publicly and privately held redwood forests, improved methodology could reduce the impact of forest management both by minimizing disturbance and diminishing propagule pressure on forested lands.

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MORPHOLOGICALLY CRYPTIC SPECIES WITHIN *DOWNINGIA YINA* (CAMPANULACEAE)

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ABSTRACT

The *Downingia yina* species complex (Campanulaceae), centered in northern California and southern Oregon, currently contains three morphologically distinguished species: *D. yina*, *D. elegans*, and *D. bacigalupii*. This complex of species is notable for high levels of morphological and cytological variation, with chromosome counts of $n = 6$, 8, 10, and 12. Molecular evidence suggests three main clades within this complex, corresponding more with cytological variation than with morphological variation. Additionally, the molecular evidence suggests a phylogeographic pattern associated with the Cascade Ranges, where members of the clade characterized by chromosome counts of $n = 6$, 8, and 10 are distributed primarily to the west of the Cascades while members of the clade characterized by chromosome counts of $n = 12$ are distributed primarily to the east. A third clade characterized by $n = 10$ is localized in the Lake of the Woods region of southern Oregon. Evidence from morphological, cytological, interfertility, and molecular data was used to re-examine the delimitation of species within this complex. *Downingia elegans* and *D. bacigalupii* are maintained, while *D. yina* is split into three morphologically cryptic species (*D. yina*, *D. willamettensis*, *D. pulcherrima*) that do not form a clade.

Key Words: Campanulaceae, chromosome races, cryptic species, *Downingia*, phylogeography.

The *Downingia yina* species complex is a monophyletic group (Schultheis 2001) comprising *D. yina* Applegate, *D. bacigalupii* Weiler, and *D. elegans* (Lindl.) Torr. The species complex represents a cytologically and morphologically variable group centered in northern California and southern Oregon. Chromosome numbers within the complex include $n = 12$ in *D. bacigalupii*, $n = 10$ in *D. elegans*, and races of $n = 6$, 8, 10 and 12 in *D. yina* (Weiler 1962; Foster 1972; Lammers 1993). Morphologically, both *D. bacigalupii* and *D. elegans* are distinguished from *D. yina* by an exserted staminal column with a sharp bend between the anthers and filament, and by the concave oval-shaped lower corolla lip with relatively parallel corolla lobes. *Downingia bacigalupii* can be distinguished from *D. elegans* by the corolla's lighter shade of purple and by the yellow pigmentation in the corolla throat, a feature also found in *D. yina*.

Morphological variation within *D. yina* has led some workers to recognize additional species or infraspecific taxa. *Downingia yina* was described by Applegate (1929) from a localized region of the southern Cascade Ranges in Klamath Co., Oregon. Shortly thereafter, Peck (1934, 1937) described two additional larger flowered species: *D. willamettensis* Peck from the Willamette Valley of Oregon, and *D. pulcherrima* Peck from eastern Oregon. In the first monograph of the genus, McVaugh (1941) recognized *D. yina* and *D. willamettensis*, including *D. pulcherrima* in the latter. McVaugh noted (1941), however, that *D. yina* and *D. willamettensis* were not readily distinguishable, and ultimately treated them as varieties within *D. yina*, var. *yina* and var. *major* McVaugh, respectively (McVaugh 1943). He distinguished the two varieties based on fruit characteristics (fusiform with hyaline lines in var. *yina*; subulate without hyaline lines in var. *major*), plant stature (larger and more erect in var. *major*), and geographic location of the populations. Weiler (1962) found that the differences described between *D. yina* and *D. willamettensis* were not maintained under greenhouse conditions. He accordingly recognized only *D. yina* with no infraspecific taxa, although noting that fresh material of *D. pulcherrima* was not examined. Weiler (1962) also noted that individuals of *D. yina sensu lato* tended to be decumbent to the west of the Cascade Ranges, and erect to the east. Foster (1972) was unable to find consistent morphological differences to correspond with cytological races in *D. yina*, but did note an ecological trend. She observed that *D. yina* chromosome races $n = 6$, 8, and 10 are found in habitats characterized by Küchler (1964) as Oregon-oak woodland or cedar-hemlock-Douglas fir mosaic while the *D. yina* chromosome race $n = 12$ is found in California mixed evergreen forest and juniper-steppe woodland, as characterized by Küchler (1964). Both Foster (1972) and Ayers (1993) followed Weiler (1962) in recognizing only *D. yina*.

The present study emerged largely from a systematic investigation of the genus *Downingia*, in which molecular data unexpectedly suggested the existence of morphologically cryptic lineages within *D. yina* (Schultheis 2001), corresponding in part to infraspecific taxa previously recog-

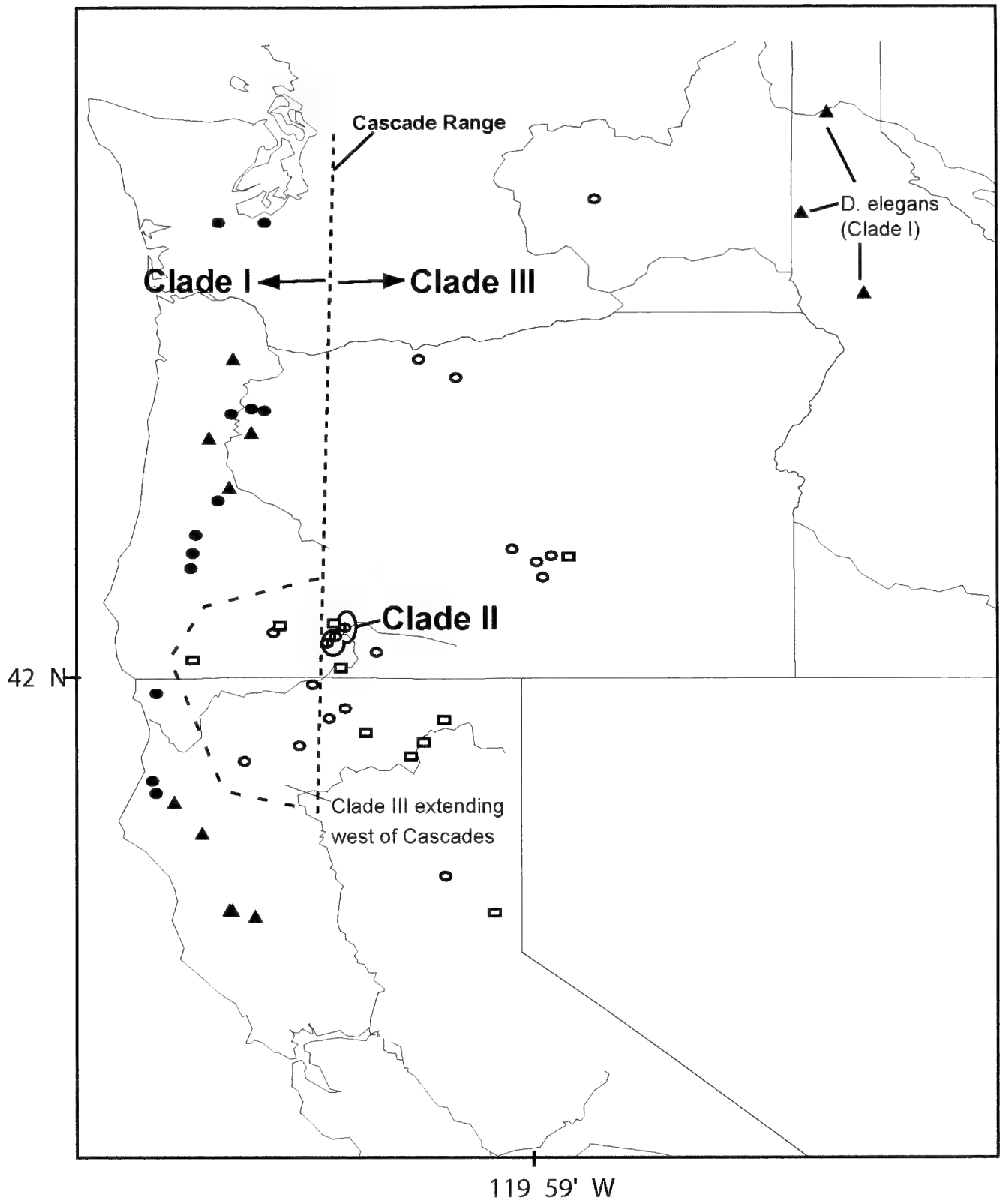

FIG. 1. Map of the northwestern USA showing localities of samples included in this study. Each symbol may represent one or multiple samples from the vicinity. The dashed line roughly corresponds to the geographic barrier created by the Cascade Range. Triangles = *Downingia elegans*. Squares = *D. bacigalupii*. Circles = samples previously included in *D. yina*. Filled circles = now assigned to *D. willamettensis*. Open circles = now assigned to *D. pulcherrima*. Circles with line through center = now assigned to *D. yina sensu strictu*. Clade I, Clade II and Clade III refer to clades identified in phylogenetic analyses.

nized. The situation was further complicated (Schultheis 2001) by the apparent para- or polyphyly of *D. yina* with respect to *D. elegans* and *D. bacigalupii*. The *D. yina* species complex thus represents a mixture of morphologically cryptic and morphologically distinctive lineages that may not correspond to the species currently recognized (Ayers 1993). The aim of this study was to further investigate the relationships and circumscriptions of *D. bacigalupii*, *D. elegans* and *D. yina* using morphological data, additional nuclear and chloroplast molecular sequence data to supplement Schultheis (2001), and available cytological and interfertility data (Weiler 1962; Foster 1972).

METHODS

Taxon Sampling

Collections were made from throughout the range of *D. elegans*, *D. bacigalupii*, and *D. yina* (Appendix 1; Fig. 1). Herbarium collections provided important supplemental material. *Downingia bicornuta* A.Gray, *D. concolor* E. Greene, *D. cuspidata* (E. Greene) Rattan, *D.*

TABLE 1. CHARACTERS USED IN MORPHOLOGICAL ANALYSES OF THE *DOWINGIA YINA* COMPLEX. Characters 1–10 are quantitative and measured in millimeters, characters 11–14 are qualitative, and characters 15–19 are ratios. [1] Characters used in cladistic analyses, with character states noted in brackets.

Character number	Character	Character definition and how assessed
1.	sepal	dorsal sepal, length
2.	back slit	corolla base to dorsal slit, length; equivalent to height of corolla tube along dorsal surface
3.	side slit	corolla base to lateral slit, length; equivalent to height of corolla tube along lateral surface
4.	upper lobe	upper corolla lobes, length
5.	lower lobe	lower corolla lip, length
6.	filament[1]	filament tube, length (<6 mm [0], >6 mm [1])
7.	anther	anther tube, length
8.	anther angle[1]	angle between anther and filament tubes (<50 [0], >70 [1])
9.	lower angle	angle of divergence between lobes of the lower corolla lip
10.	horns[1]	anther horns, length (<0.62 mm [0], >0.62 mm [1]); refers to triangular projections on each of the two smaller anthers
11.	anther back	trichomes on anther dorsal surface: abundant (0), few (1), none (2)
12.	upper lobe orientation	upper corolla lobes, orientation: parallel (0), intermediate (1), divergent (2)
13.	yellow[1]	yellow on lower corolla lobe: present (0), absent (1)
14.	lower lobe shape	lower corolla lobe, shape: acute (0), intermediate (1), mucronate (2)
15.	filament/anther	filament length/Anther length
16.	sideslit/backslit[1]	length of lateral slit/Length of dorsal slit (≥0.6 [0], <0.6 [1])
17.	filament/backslit[1]	filament length/Length of dorsal slit (≤1 [0], 1–2 [1], >2 [2])
18.	upper/lower lobe	upper lobes length/Lower lip length
19.	backslit/upper lobe	length of dorsal slit/Upper lobes length

montana (E. Greene) Rattan, and *D. ornatissima* E. Greene were chosen as outgroup taxa based on previous phylogenetic analyses within the genus (Schultheis 2001).

Molecular

Generation of sequence data. Extraction of total DNA from 24 samples first reported in Schultheis (2001) and 9 new samples (Appendix 1) involved use of either the CTAB protocol of Doyle and Doyle (1987) or Hillis et al. (1996) with minor modifications (Schultheis 2001), or use of Qiagen DNeasy Plant mini kits following manufacturer's instructions. Most plant tissue samples were stored in a cooler while in the field and transferred to a −80C freezer within one week of collection. Voucher specimens were either the same plant from which tissue for DNA extraction was taken, or were other plants from the same site.

Sequence data were generated from the nuclear 18S–26S rDNA internal transcribed spacer (ITS) and the chloroplast 3′*trn*K intron. Amplification and sequencing methods changed during the course of the project, as new techniques became available. Single-stranded DNAs of ITS 1 and ITS 2 were generated, purified, and manually sequenced following Baldwin (1992). Double-stranded DNAs of ITS 1, ITS 2 and the 3′*trn*K intron were generated, purified, and sequenced using automated sequencing technology following Schultheis (2001). Sequences are deposited in Genbank.

Sequence alignment. All alignments were visual. Sites coded with "?" or with an IUPAC-IUB ambiguity code represent basepairs where sequence produced with neither primer produced a sufficiently strong or clear signal for confident basepair assignment. Indels, coded as "-", were treated as missing data. Two regions were excluded from the ITS dataset due to ambiguous sequence alignment (positions 132–138 and 284–291 of the aligned ITS data set).

Evaluation of sequence data. Separate and combined analyses using a parsimony criterion were conducted for ITS and 3′*trn*K intron data. All analyses employed heuristic searches with 10,000 replicates of random sequence addition and tree-bisection-reconnection (TBR) branch swapping. Conservative estimates of clade support were assessed using 10,000 replicates of the "fast" bootstrap option in PAUP 4.0b5. Decay analyses (Donoghue et al. 1992; Bremer 1994) using Autodecay (Eriksson 1998) were conducted for the 3′*trn*K intron and the combined molecular analyses.

Morphology

Fresh and/or herbarium material was examined from 80 localities (Appendix 1, Fig. 1) and 450 flowers. Nineteen characters were included for phenetic analyses, including 10 quantitative, 4 qualitative, and 5 ratio characters (Table 1). All

characters are floral, because vegetative characters are not generally useful for distinguishing species of *Downingia*. Characters were observed or measured against a ruler under a dissecting scope, except for anther horn length which was measured with an ocular micrometer.

Morphometric analyses. Analyses of variance were conducted to identify characters differing significantly among the three currently recognized species, and Tukey tests were used to identify which species differed. The same was done within *D. yina* for the three groups identified by molecular analyses (see results). For multivariate analyses, a data matrix was created containing the average value for each character from each collection locality. Multivariate analyses included cluster analysis using Euclidean distances and single linkage, discriminant function analysis, and Principal Components Analysis (PCA), the latter using a matrix standardized so that each character had a mean of zero and a standard deviation of one. All statistical analyses were performed with SYSTAT 5.2.1.

Cladistic analyses. One qualitative and five quantitative characters (indicated in Table 1) were used in a cladistic analysis of the 26 populations for which molecular data were also available, plus one population per outgroup taxon. Phylogenetic analyses using a parsimony criterion were conducted with PAUP 3.1.1 (Swofford 1993) or PAUP *4.0b5. The analysis employed a heuristic search with 100 replicates of random taxon addition and TBR branch-swapping. Qualitative characters excluded from the analysis were polymorphic within most populations. Character states for the quantitative characters were determined by searching for gaps within the character distribution among specimens that were greater than 2 times the average population standard deviation (Archie 1985). Most quantitative characters were excluded from the cladistic analysis because no character states could be defined. The character "locule", referring to the number of locules in the ovary, separates the *D. yina* complex from the outgroup taxa. The morphological data matrix is provided in Table 2.

Cytology

Chromosome counts were obtained from unpublished theses (Weiler 1962; Foster 1972) and from numerous specimens deposited at the UC and JEPS herbaria as chromosome vouchers (Appendix 1). Chromosome number was treated as an ordered character. All known chromosome counts for *D. bacigalupii*, *D. elegans*, *D. bicornuta*, *D. cuspidata*, *D. ornatissima* and *D. montana* report a single number for each of the species (Wood 1961; Foster 1972; Weiler 1962; Lammers 1993). All samples of these taxa were scored based on chromosome counts reported for the species, regardless of whether a count was obtained from the population sampled here. The only exception is *D. bacigalupii* sample 585-99, which was scored as unknown since the population is at the limits of the species range, and no chromosome counts were available from the vicinity. Chromosome counts for *D. concolor* are $n = 8$ and $n = 9$ (Weiler 1962; Lammers 1993). The samples of *D. concolor* included here fall within the known geographic range of $n = 9$ reports for *D. concolor* (Weiler 1962), and were scored as such. Populations of *D. yina* were scored based on the geographic proximity of the population to a population with a documented chromosome number (indicated in Table 2; Weiler 1962; Foster 1972). MacClade version 3.0 (Maddison and Maddison 1992) was used to reconstruct the most parsimonious chromosome numbers characterizing each node on trees produced from the combined analysis of the ITS and 3′*trn*K datasets.

Analyses of Combined Molecular, Morphological, and Cytological Data

A partition-homogeneity test (Farris et al. 1995) performed in PAUP *4.0 (Swofford 2001) confirmed combinability of the ITS plus 3′*trn*K data ($P = 0.247$; 1000 replicates, heuristic searches with random addition and TBR branch swapping), and of the molecular data with the morphological and cytological data ($P = 0.094$). Morphological and cytological data were combined as a single partition for the test since cytological data consisted of only one character (chromosome number). A branch and bound search of the combined data was conducted under a parsimony criterion. Clade support was assessed using 10,000 replicates of the "fast" bootstrap option. The morphological data came from the same or neighboring populations as the sequence data (Table 2). The cytological data consisted of chromosome numbers and did not include information regarding meiotic configurations of chromosomes in hybrid plants.

Interfertility

Information regarding interfertility and crossability among members of the *D. yina* complex comes from Weiler's unpublished thesis (1962), in which he documented the results of numerous interspecific crosses within *Downingia*. His data include qualitative assessments of seed set, germination, and hybrid condition (e.g., flowering, green, chlorotic, dying in seedling stage), some quantitative assessments of pollen stainability, and analysis of meiotic configurations.

Information regarding interfertility and crossability within *D. yina* comes from Foster's

TABLE 2. MORPHOLOGICAL DATA MATRIX USED FOR CLADISTIC ANALYSES. Sample numbers correspond to Appendix 1, with the following prefixes: B = *Downingia bacigalupii*, E = *D. elegans*, Y = *D. yina*, M = *D. montana*, C = *D. concolor*, O = *D. ornatissima*, BI = *D. bicornuta*, CU = *D. cuspidata*. Characters and states are listed in Table 1. The "locule" character refers to the number of locules in the ovary [bilocular (0), unilocular (1)]. For *D. yina* the "chromosome" character refers to the chromosome number based on reports or vouchers from the same or a neighboring population, indicated in parentheses. This character was included in the analyses of all data combined, but was not included in the analysis of morphological data alone. For some samples, the morphological data were combined with the molecular data from a neighboring population, indicated in parentheses.

Sample	Character							
	6	8	10	13	16	17	Locule	Chromosome
B *Schultheis 585-99*	0	1	1/0	0	0	1	1	?
B *Schultheis 240-95*	1	1/0	1/0	0	0	1	1	12
B *Schultheis 237-95*	1	1	0	0	0	1/2	1	12
B *Schultheis 231-95*	1	1	1/0	0	0	1	1	12
B *Schultheis 251-95*	1	1	0	0	1/0	1/2	1	12
E *Schultheis 243-95*	1	1	0	1	1	1	1	10
E *Schultheis 242-95*	0	1	0	1	1	1/0	1	10
E *Schultheis 320-96*	0	1	0	1	1/0	1	1	10
E *Weiler 60138* (Foster 70-15-4)	0	1/0	0	1	0	0	1	10
Y *Oswald & Ahart 3943*	0	0	0	0	1/0	1	1	?
Y *Schultheis 247-95*	0	0	0	0	0	0	1	10 (Foster 70-96-11)
Y *Tracy 3217*	0	0	0	0	0	0	1	10 (Foster 70-96-11)
Y. *T. Obrien s.n.*	0	0	0	0	0	0	1	?
Y *Schultheis 236-95*	0	0	0	0	0	0	1	12 (Weiler 60207)
Y *D. Barbe 348*	0	0	0	0	0	1/0	1	12 (Foster, Siskiyou)
Y *Schultheis 241-95*	0	0	1/0	0	0	0	1	12 (Foster, Harney)
Y *Schultheis 584-99*	0	0	0	0	0	0	1	12 (Foster 70-43-15)
Y *Schultheis 245-95*	0	0	0	0	0	1/0	1	12 (Foster 70-43-15)
Y *Weiler 61449*	0	0	0	0	0	0	1	10 (Weiler 61200)
Y *Schultheis 581-99*	0	0	0	0	0	0	1	10 (Weiler 61451)
Y *R. Bacigalupi 7978*	0	0	0	0	0	0	1	10 (Weiler 61200)
Y *Cook 962*	0	0	0	0	0	0	1	8
Y *Peck 16291* (Foster 68-210)	0	0	0	0	0	0	1	6 (Foster 68-210)
Y *Schultheis 319-95*	0	0	0	0	1/0	0	1	12 (Weiler 61383)
Y *Weiler 61333* (Foster 68-51)	0	0	0	0	0	0	1	10
Y *R. Bacigalupi 7894*	0	0	0	0	0	0	1	12 (Foster,Wasco)
BI *Schultheis 100-95*	0	0	1/0	0	0	0	0	11
C *Schultheis 195-95*	0	0	0	0	0	1/0	0	9
M *Schultheis 235-95*	0	0	0	0	0	0	1	11
CU *Schultheis 179-95* (197-95)	0	0	0	0	0	0	0	11
O *Schultheis 180-95*	0	0	0	0	0	1	0	12

unpublished thesis (1972). She documents meiotic configurations and pollen stainability for crosses between individuals of the same and different chromosome races. I assigned each of Foster's parent populations to a molecular clade, based either on sequence data from her voucher specimens, or on close proximity of the vouchered population to a population with sequence data. I applied an ANOVA to Foster's raw pollen stainability data to examine whether there were significant decreases in stainability in hybrids between versus within chromosome races, and between versus within molecular clades.

RESULTS

Cladistic Analyses

Levels of divergence for the ITS dataset ranged from 0.0 to 0.017, excluding outgroups. Analysis of ITS data resulted in 104 minimum-length trees based on 43 parsimony-informative characters (length = 100; CI = 0.90, 0.83 without uninformative characters; RI = 0.91). Levels of divergence for the 3′*trn*K dataset ranged from 0.0 to 0.027, excluding outgroups. Analysis of the 3′*trn*K dataset resulted in 42 trees based on 19 parsimony-informative characters (length = 67; CI = 0.94, 0.83 without uninformative characters; RI = 0.89). Combined molecular analyses produced 2 trees based on 43 parsimony-informative characters (length = 158; CI = 0.91, 0.77 without uninformative characters; RI = 0.89). Combined molecular, morphological and cytological analyses produced 72 trees based on 50 parsimony-informative characters (length = 176; C.I. = 0.87, 0.72 without uninformative characters; RI = 0.86).

All analyses (ITS dataset; 3′*trn*K dataset; combined molecular datasets; combined molecu-

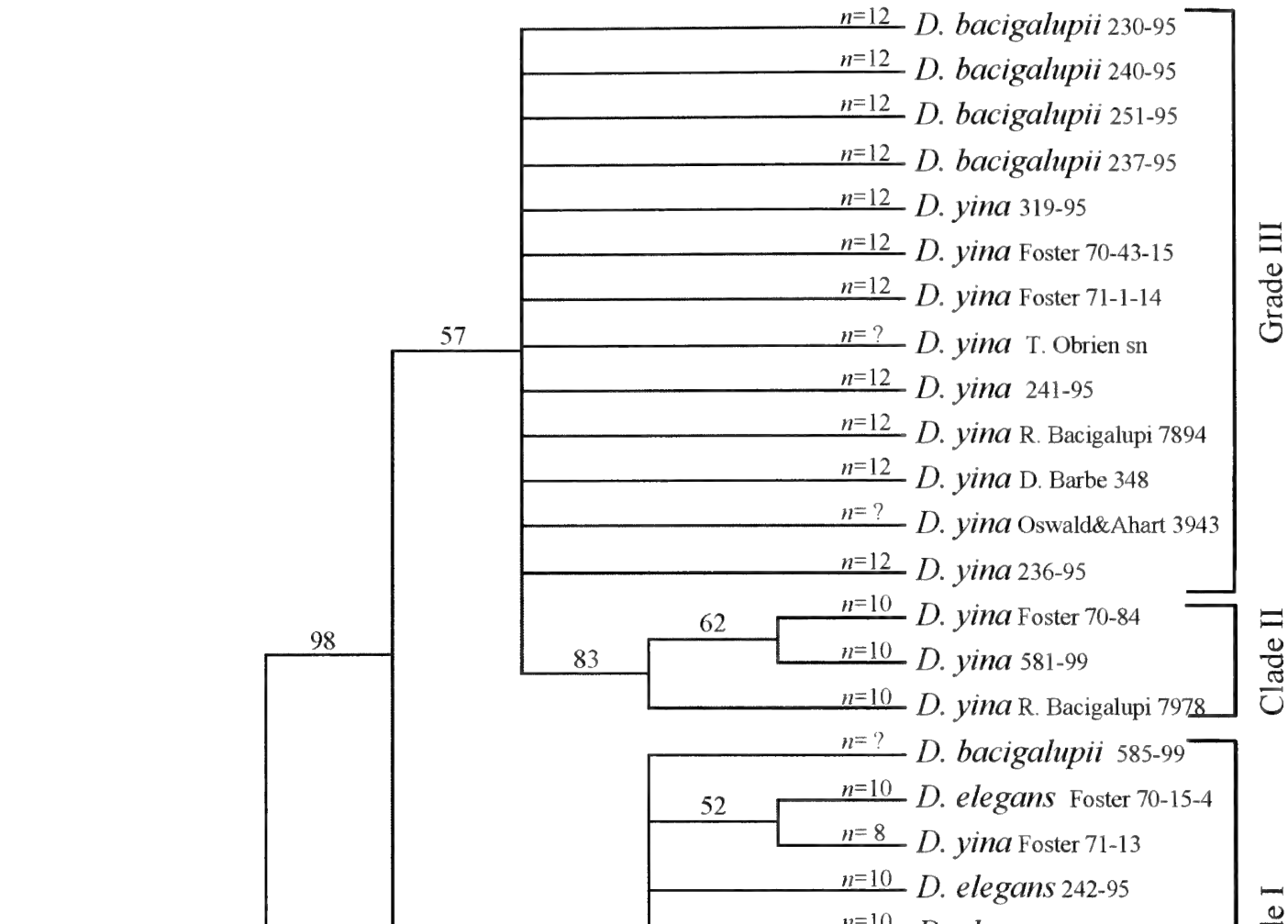

FIG. 2. The strict consensus of 104 minimum-length ITS parsimony trees (length = 100; C.I. = 0.90, 0.83 w/o uninformative characters; R.I. = 0.91) produced from a heuristic search with 10,000 replicates of random taxon addition and TBR branch swapping. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

lar, morphological and cytological datasets) except that of morphological data alone resulted in three main clades or grades (Figs. 2–5). Clade I comprised *D. elegans* and *D. yina* pro parte. Clade II comprised *D. yina* pro parte. Clade III comprised *D. bacigalupii* and *D. yina* pro parte. Primary differences among the trees produced from different analyses were the following: (1) There was a sister relationship between Clades I and II in trees resulting from analyses of all datasets but the ITS dataset, in which Clade II is aligned with grade III (Fig. 2). (2) *Downingia elegans* sample Foster 70-15-4 was resolved as part of Clade I in all trees except those resulting from analysis of the 3′*trn*K dataset, in which it fell in an unresolved position between Clades I and II (Fig. 3). This sample is from Snow Mountain, in Lake Co., California, at the southern limit of the species range (Fig. 1). (3) *Downingia bacigalupii* sample 585-99 is aligned with Clade I in ITS trees (Fig. 2), but is sister to other members of Clade III in all other trees. Sample 585-99 is from Josephine Co., Oregon, at the western periphery of the species range

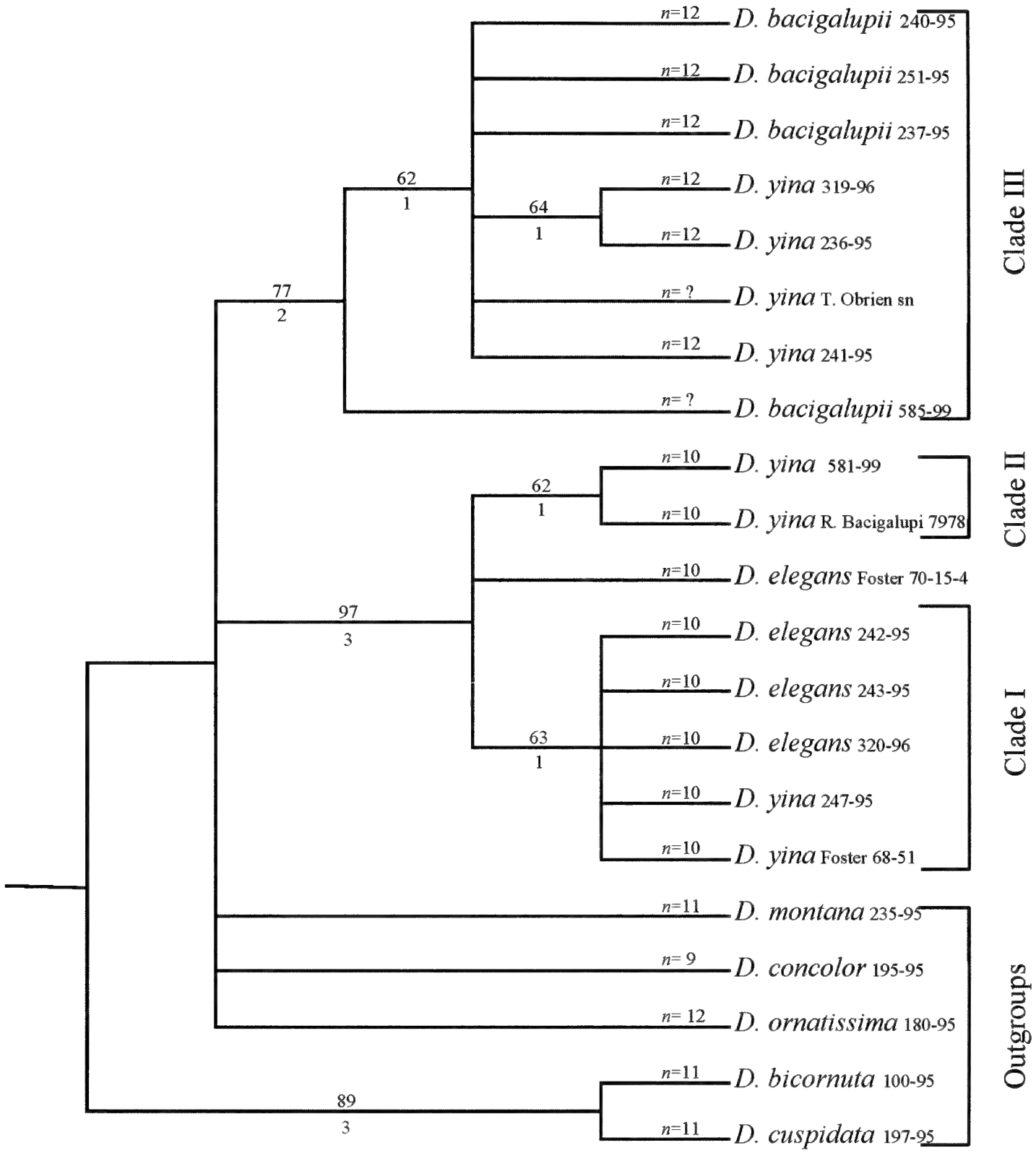

FIG. 3. The strict consensus of 42 minimum-length 3′*trn*K intron trees (length = 67; C.I. = 0.94, 0.83 w/o uninformative characters; R.I. = 0.89) produced from a heuristic search with 10,000 replicates of random taxon addition and TBR branch swapping. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Numbers below the branches are decay indices. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

(Fig. 1). (4) When morphological and cytological data are combined with molecular data, the resulting trees resolve samples of *D. elegans* and of *D. bacigalupii* as clades within Clade I and Clade III respectively (Fig. 5). *Downingia bacigalupii* sample 585-99, however, is resolved as sister to Clade III, and *D. elegans* sample Foster 70-15-4 is unresolved within Clade I.

The strict consensus of 2556 trees based on six parsimony-informative characters (length = 9, C.I. = 0.67, RI = 0.88) produced by the cladistic analysis of only the morphological data showed no resolution (figure not shown).

Morphometric Analyses

Downingia yina complex. Univariate analyses within the *D. yina* complex showed that the three currently recognized species were significantly different from one another for numerous characters, although ranges overlapped for all characters (Table 3). Anther angle and the angle of

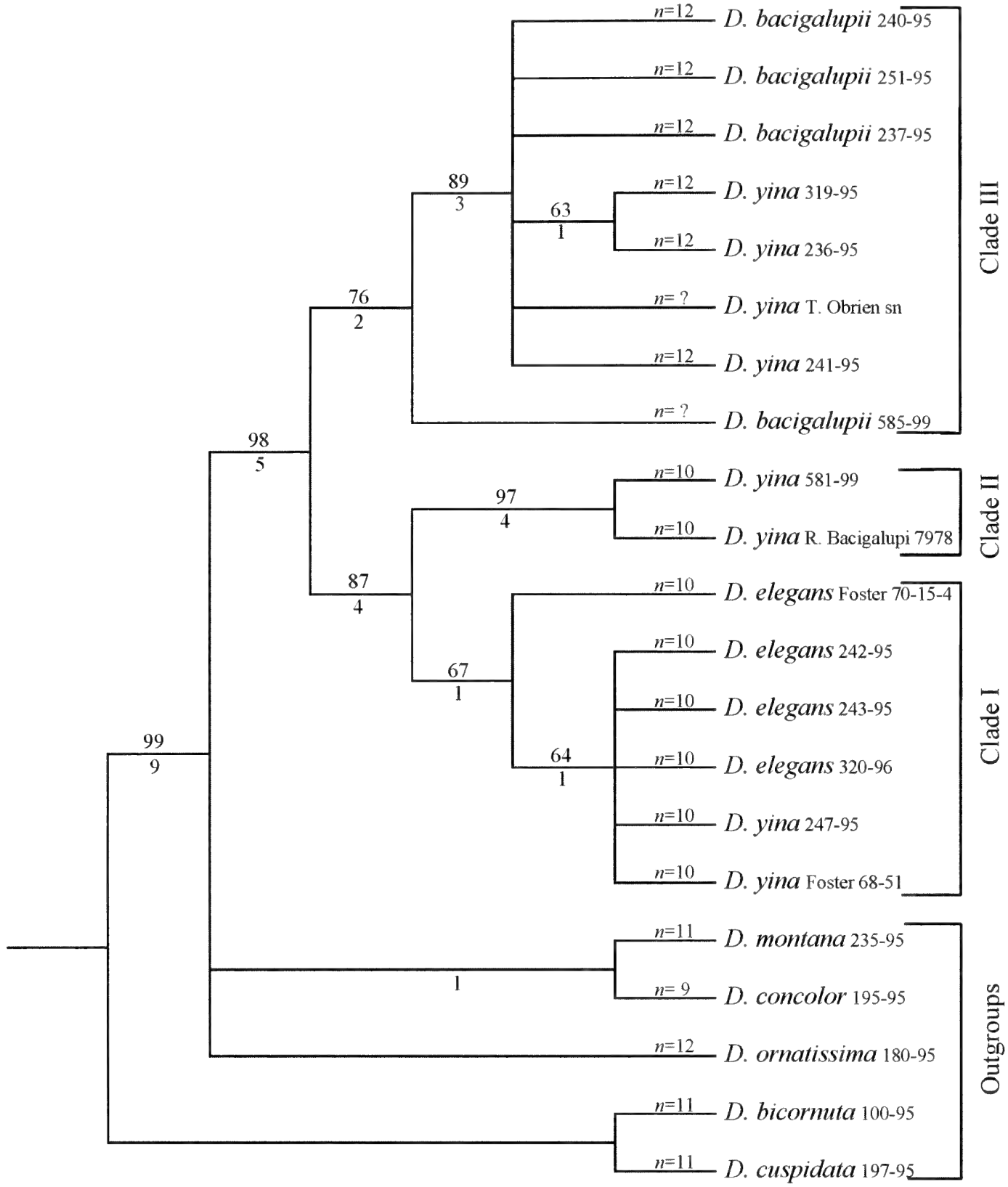

FIG. 4. The strict consensus of two minimum-length trees (length = 158; C.I. = 0.91, 0.77 w/o uninformative characters; R.I. = 0.89) from a heuristic search of combined ITS and 3′*trn*K intron data, with 10,000 replicates of random taxon addition and TBR branch swapping. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Numbers below the branches are decay indices. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

divergence between lobes of the lower corolla lip in particular distinguished *D. elegans* and *D. bacigalupii* from *D. yina*. The former two taxa had more sharply bent anthers and less divergent lower corolla lobes than *D. yina*. The filament of *D. bacigalupii* was longer on average than that of *D. elegans* and *D. yina*. Additionally, *D. elegans* could be distinguished by the lack of yellow pigmentation on the lower corolla lip.

PCA analyses of all samples using all data showed clear separation among *D. elegans*, *D. bacigalupii*, and *D. yina*, particularly when principal components I and III were plotted (Fig. 6). This separation was also clear when only ratio characters were used or when ratio characters were excluded. Characters of particular importance in the PCA analyses were the anther angle, the filament/back slit ratio, and the angle of divergence between the lower corolla lobes. The percent of total variance explained by components I, II, and III was 45.2%, 17.5%, and 12.0%, respectively.

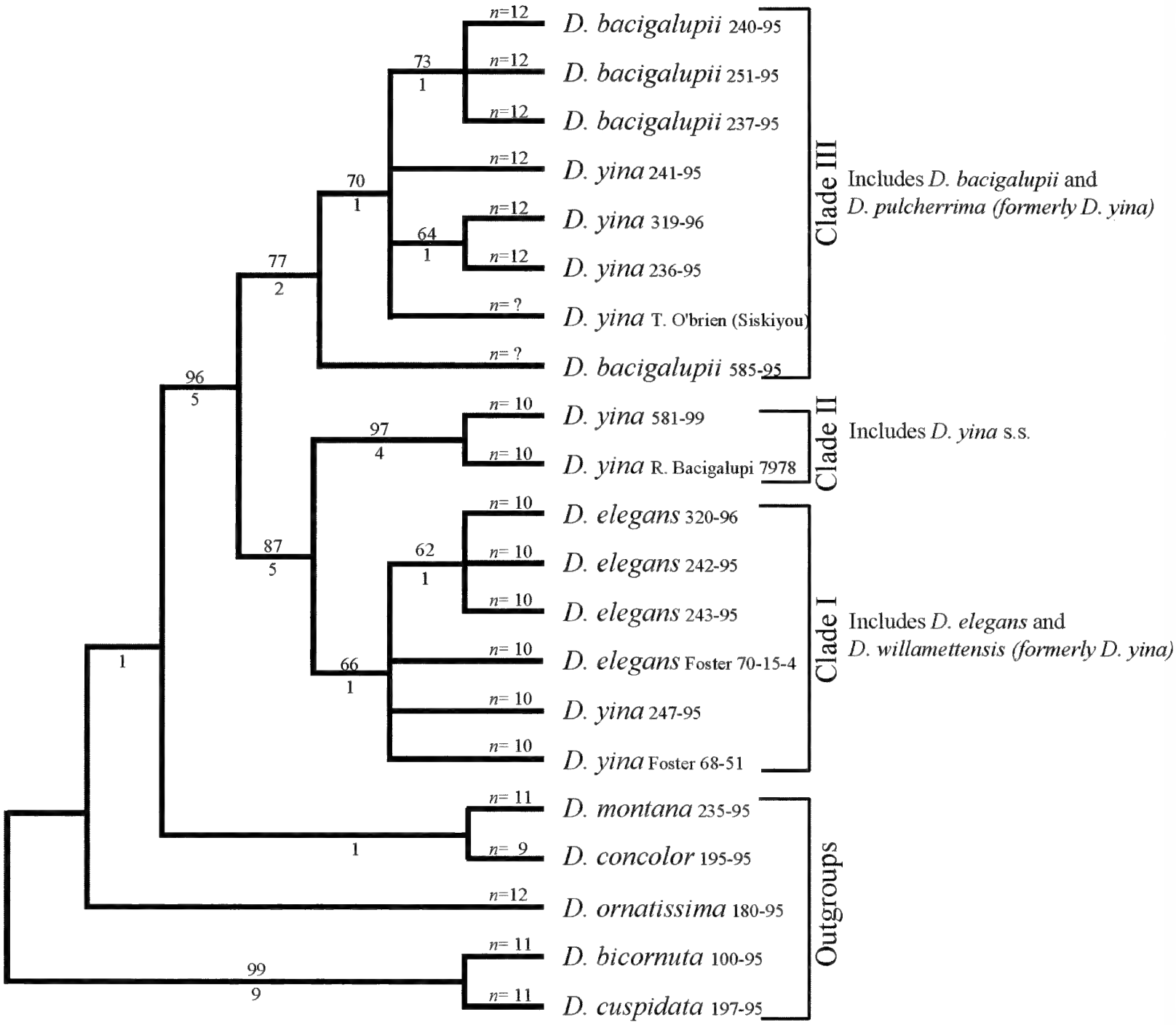

FIG. 5. The strict consensus of 72 minimum-length trees (length = 176; C.I. = 0.87, 0.72 w/o uninformative characters; R.I. = 0.86) produced from a branch-and-bound search of combined data. The data matrix included the ITS, 3′*trn*K, morphological, and cytological data. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Numbers below the branches are decay indices. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

Cluster analysis (not shown) produced two main groups, one with *D. yina* samples and the other with a mixture of *D. elegans* and *D. bacigalupii* samples. One sample of *D. elegans* (*Ehlers & Erlanson 39*) and one sample of *D. bacigalupii* (582-99) together joined at the base of the *D. yina* cluster.

Variation within Downingia yina. Univariate analyses revealed that significant character differences were evident between *D. yina* samples from the three main molecular clades, but with overlapping ranges (Table 4). No qualitative characters could be used to uniquely identify the three groups. In general, Clade II samples tended to be smaller for most quantitative characters measured (Table 4). Samples from Clade I tended to have a less sharply bent anther and a wider angle of divergence between the lobes of the lower corolla lip than did samples from Clades II and III.

PCA analyses of only *D. yina* samples using all data did not clearly distinguish between samples from Clades I, II, and III (not shown). Discriminant function analysis of *D. yina* samples showed better separation of the three groups, but with areas of overlap (Fig. 7). Characters of particular importance in the discriminant function analyses were anther length, the angle of divergence between the lobes of the lower corolla lip, and trichome density on the dorsal anther surface.

Cluster analysis (not shown) grouped all of the *D. yina* samples together, but did not resolve groups corresponding to Clade I, II, and III samples.

TABLE 3. UNIVARIATE STATISTICS FOR THE *DOWNINGIA YINA* COMPLEX. Means, standard deviations and ranges (in parentheses) are provided for each character within each species. Superscripts indicate groups that are significantly different from one another using Tukey multiple comparison tests following ANOVA. Groups with no superscript or that share a superscript are not significantly different.

Character	*D. elegans* (n = 62)	*D. yina* (n = 317)	*D. bacigalupii* (n = 69)
Sepal (mm)	5.31 ± 1.30^{A} (3.0–8.0)	4.75 ± 1.28^{B} (0.50–10.0)	5.81 ± 1.80^{A} (3.0–10.0)
Side slit (mm)	2.33 ± 0.45^{A} (1.5–3.0)	4.10 ± 0.71^{B} (1.75–6.0)	2.96 ± 0.54^{C} (2.0–4.25)
Back slit (mm)	4.48 ± 0.75^{A} (3.0–6.0)	4.80 ± 0.89^{B} (2.25–7.5)	3.87 ± 0.91^{C} (2.25–6.0)
Upper lobe (mm)	4.04 ± 1.16^{A} (2.0–7.0)	3.71 ± 0.93^{A} (2.0–6.75)	6.75 ± 1.35^{B} (4.0–11.0)
Lower lobe (mm)	6.53 ± 1.82^{A} (3.5–12.0)	6.18 ± 1.30^{A} (3.0–9.5)	8.40 ± 1.80^{B} (5.0–14.0)
Filament (mm)	5.27 ± 1.48^{A} (2.5–7.5)	3.17 ± 0.92^{B} (1.25–5.75)	7.48 ± 1.50^{C} (3.25–9.75)
Anther (mm)	2.68 ± 0.46^{A} (1.5–3.5)	2.18 ± 0.36^{B} (1.25–3.0)	2.89 ± 0.42^{C} (1.25–3.75)
Anther angle (degrees)	84.07 ± 14.40^{A} (28.0–90.0)	22.18 ± 9.11^{B} (0.0–51.0)	88.80 ± 6.75^{A} (38.0–90.0)
Lower angle (degrees)	9.16 ± 11.92^{A} (0.0–50.0)	53.69 ± 12.79^{B} (20.0–90.0)	12.31 ± 9.88^{A} (0.0–30.0)
Horns (mm)	0.44 ± 0.08^{A} (0.26–0.75)	0.41 ± 0.11^{A} (0.13–0.79)	0.60 ± 0.10^{B} (0.32–0.86)
Filament/anther	1.95 ± 0.34^{A} (1.2–2.55)	1.45 ± 0.30^{B} (0.625–2.375)	2.58 ± 0.35^{C} (1.6–3.6)
Side slit/back slit	0.53 ± 0.10^{A} (0.35–1.0)	0.86 ± 0.09^{B} (0.47–1.3)	0.79 ± 0.13^{C} (0.5–1.11)
Filament/back slit	1.18 ± 0.23^{A} (0.56–1.75)	0.66 ± 0.18^{B} (0.38–2.0)	1.97 ± 0.36^{C} (1.42–3.56)
Upper lobe/lower lobe	0.64 ± 0.15^{A} (0.25–1.0)	0.61 ± 0.14^{A} (0.31–1.28)	0.81 ± 0.14^{B} (0.54–1.14)
Back slit/upper lobe	1.19 ± 0.35^{A} (0.6–2.22)	1.37 ± 0.41^{B} (0.5–3.11)	0.59 ± 0.18^{C} (0.35–1.14)

Cytology

Chromosome numbers within the *Downingia yina* complex appear to correspond to the molecular clades identified with ITS and 3′*trn*K sequences (Figs. 2–4, Appendix 1). All samples in molecular Clade I for which chromosome counts were available were $n = 10$ in *D. elegans* and $n = 6$, 8 or 10 in *D. yina*. *Downingia yina* counts of $n = 6$ and $n = 8$ were documented from Marion and Lane counties in Oregon, respectively (Weiler 1962; Foster 1972). All samples in molecular Clade II were *D. yina* with $n = 10$. All samples in molecular Clade III were $n = 12$ in either *D. yina* or *D. bacigalupii*. Character state reconstruction suggests an ancestral state of $n = 10$ in Clades I and II, and an ancestral state of $n = 12$ in Clade III. The ancestral state for the entire *D. yina* complex is equivocal.

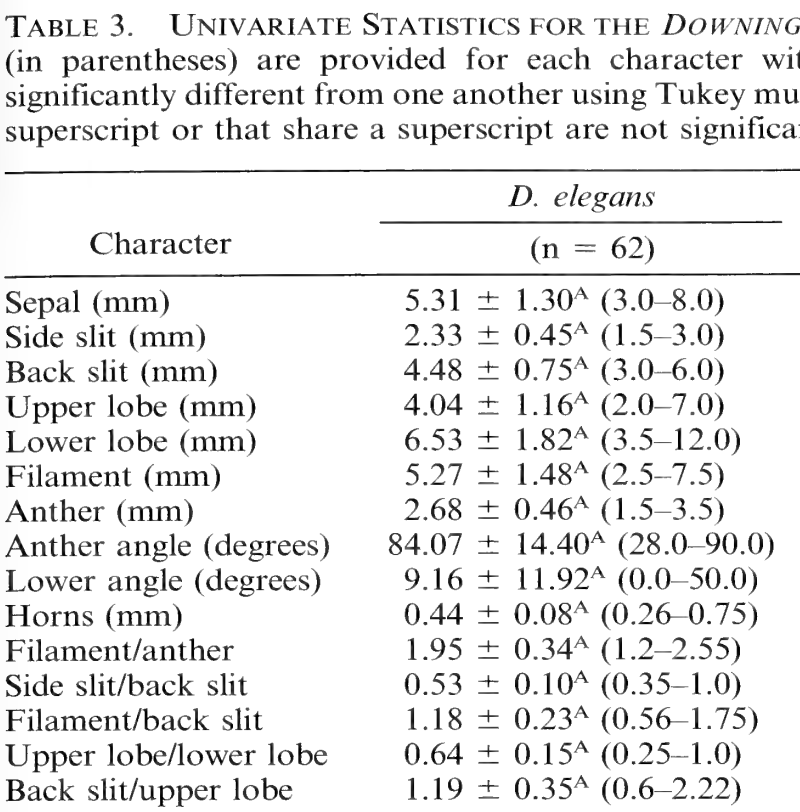

FIG. 6. Plot of principal components one and three using the characters listed in Table 1. Filled symbols represent Clade I. Symbols with a line through the center represent Clade II. Open symbols represent Clade III. Triangles = *Downingia elegans*. Squares = *D. bacigalupii*. Circles = *D. yina*.

Interfertility

Foster's results (1972) show that within *D. yina*, crosses between populations with different chromosome numbers showed a significant reduction in pollen stainability relative to crosses between populations with the same chromosome numbers (Table 5; Foster 1972). Similarly, pollen stainability was significantly reduced in crosses between populations presumed to be from different molecular clades relative to those presumed to be from the same molecular clades (Table 5; Foster 1972).

Weiler's results (1962) from interspecific reciprocal crosses (Table 6) reflect Foster's results within *D. yina* in that pollen stainability and meiotic irregularities seemed to be affected more by differences in chromosome number than by species identification (*D. elegans*, *D. bacigalupii*, or *D. yina*). Crosses between *D. elegans* ($n = 10$) and $n = 10$ populations of *D. yina*, for example, produced 10 bivalents and no significant reductions in pollen stainability (Table 6), in contrast to the reduction in pollen stainability for crosses within *D. yina* but between populations of different chromosome number (Table 5; Foster 1972).

TABLE 4. UNIVARIATE STATISTICS WITHIN *DOWNINGIA YINA*. Means, standard deviations, and ranges (in parentheses) are provided for each character within inferred molecular clades. Superscripts indicate groups that are significantly different from one another using Tukey multiple comparison tests following ANOVA. Groups with no superscript or that share a superscript are not significantly different.

Character	Clade I (n = 100)	Clade II (n = 30)	Clade III (n = 187)
Sepal (mm)	4.78 ± 1.03^{AB} (3.0–7.75)	4.22 ± 0.96^{B} (0.5–6.0)	4.82 ± 1.42^{A} (2.0–10.0)
Side slit (mm)	4.18 ± 0.63^{A} (2.75–6.0)	3.40 ± 0.49^{B} (2.75–4.5)	4.17 ± 0.73^{A} (1.75–6.0)
Back slit (mm)	4.86 ± 0.78^{A}(3.25–7.0)	3.74 ± 0.53^{B} (3.0–5.0)	4.94 ± 0.88^{A} (2.25–7.5)
Upper lobe (mm)	3.41 ± 0.71^{B} (2.0–5.75)	3.44 ± 0.81^{B} (2.0–5.0)	3.90 ± 0.99^{A} (2.0–6.75)
Lower lobe (mm)	6.00 ± 0.96^{C} (3.25–8.5)	5.09 ± 0.99^{B} (3.25–6.75)	6.46 ± 1.39^{A} (3.0–9.5)
Filament (mm)	2.99 ± 0.62^{C} (2.0–4.75)	2.33 ± 0.45^{B} (1.75–3.5)	3.40 ± 1.01^{A} (1.25–5.75)
Anther (mm)	2.12 ± 0.33^{C} (1.25–2.75)	1.75 ± 0.33^{B} (1.25–2.25)	2.28 ± 0.32^{A} (1.25–3.0)
Anther angle (degrees)	18.72 ± 8.10^{B} (2.0–40.0)	22.50 ± 6.11^{AB} (7.0–33.0)	23.96 ± 9.52^{A} (0.0–51.0)
Lower angle (degrees)	59.42 ± 12.24^{B} (35.0–90.0)	46.27 ± 12.40^{A} (20.0–68.0)	52.06 ± 12.08^{A} (22.0–78.0)
Horns (mm)	0.38 ± 0.08^{B} (0.19–0.61)	0.34 ± 0.04^{B} (0.26–0.42)	0.42 ± 0.13^{A} (0.13–0.77)
Filament/anther	1.42 ± 0.23 (1.0–2.0)	1.37 ± 0.30 (0.875–2.2)	1.48 ± 0.34 (0.625–2.375)
Side slit/back slit	0.86 ± 0.08^{A} (0.62–1.07)	0.91 ± 0.09^{B} (0.77–1.25)	0.85 ± 0.10^{A} (0.47–1.31)
Filament/back slit	0.61 ± 0.08^{B} (0.5–0.9)	0.62 ± 0.08^{AB} (0.47–0.75)	0.69 ± 0.22^{A} (0.38–2.0)
Upper lobe/lower lobe	0.58 ± 0.12^{C} (0.33–0.9)	0.68 ± 0.13^{B} (0.45–0.92)	0.62 ± 0.15^{A} (0.31–1.3)
Back slit/upper lobe	1.50 ± 0.44^{B} (0.78–3.11)	1.17 ± 0.41^{A} (0.60–2.22)	1.34 ± 0.38^{A} (0.50–2.5)

DISCUSSION

The *Downingia yina* species complex currently comprises three species that are readily distinguished from one another on the basis of morphological characteristics (Weiler 1962; Ayers 1993; Fig. 6, Table 3). *Downingia elegans* and *D. bacigalupii* differ from *D. yina* in that the anthers form a sharp angle relative to the filaments, and the lower corolla lobes are relatively parallel versus divergent in *D. yina*. The chromosome numbers and the yellow patches on the lower corolla lobes readily distinguish *D.bacigalupii* from *D. elegans*. As outlined in the introduction, previous workers (Peck 1934, 1937; McVaugh 1941, 1943) recognized that *D. yina* may represent multiple taxa, which were variously named: *D. yina* Applegate, *D. yina* Applegate var. *major* McVaugh, *D. willamettensis* Peck, *D. pulcherrima* Peck. Recent molecular analyses lent merit to these interpretations, but sampling within the *D. yina* complex was very limited (Schultheis 2001). The additional molecular data presented here substantiates these patterns, and demonstrates that samples of *D. yina* fall into three separate molecular clades, with *D. elegans* and *D. bacigalupii* nested within two of these three clades (Figs. 2–4). Taken independently, this paraphyletic or polyphyletic pattern with respect to the sequence data from either the nuclear or chloroplast genomes (Figs. 2–4) might only represent gene rather than organismal phylogenies (Doyle 1992; Knox 1998). High resolution molecular data are expected to reveal patterns in which paraphyletic progenitor species (with respect to the molecular data) give rise to monophyletic derivative species (Rieseberg and Brouillet 1994; Graybeal 1995; Olmstead 1995), in this case *D.*

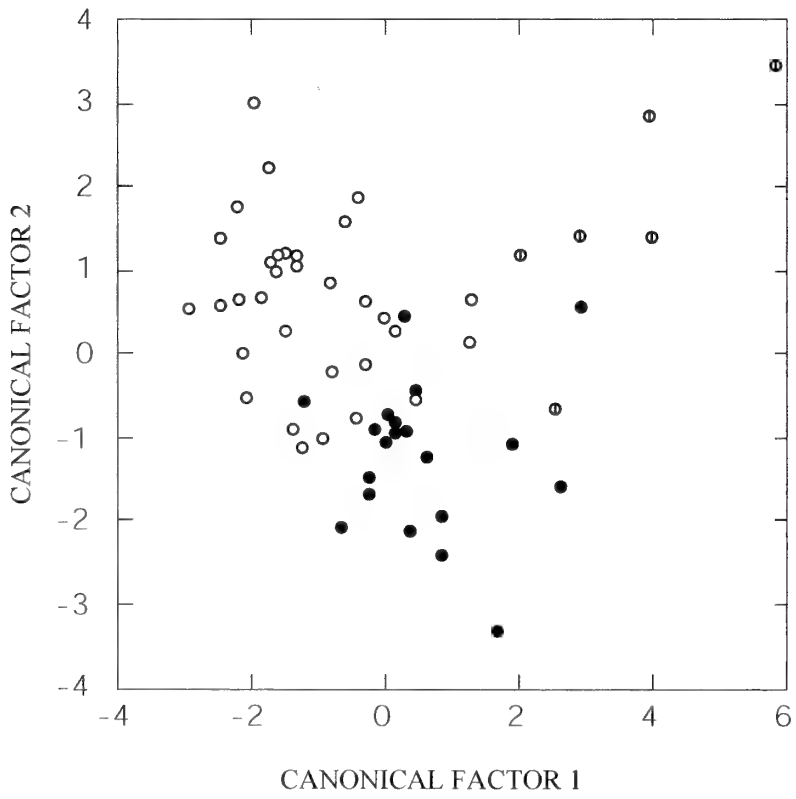

FIG. 7. Plot of canonical factors one and two from discriminant function analysis of *Downingia yina* samples using the characters listed in Table 1. Filled circles = Clade I. Circles with a line through the center = Clade II. Open circles = Clade III.

TABLE 5. MEAN PERCENT STAINABLE POLLEN IN CROSSES BETWEEN POPULATIONS OF *DOWNINGIA YINA*. Significant differences occur for populations with the same versus different chromosome numbers and for populations from the same versus different molecular clades. (Raw data taken from Foster (1972) and reanalyzed).

Cross type	Mean	SE	n	P value
Chromosome numbers same	93.4	8.4	5	0.001
Chromosome numbers differ	49.9	5.6	11	
Same molecular clade	83.0	8.3	7	0.007
Different molecular clades	48.3	7.3	9	

TABLE 6. MEAN PERCENT STAINABILITY AND MEIOTIC CONFIGURATIONS IN INTERSPECIFIC CROSSES WITHIN THE *DOWNINGIA YINA* COMPLEX. Data taken from Weiler (1962).

	D. elegans	*D. bacigalupii*
D. elegans	>95%	
$n = 10$		
D. bacigalupii	30.8–78.3%	>95%
$n = 12$	1ch3 + 9II + 1I	
D. yina	51.3–79.5%	>95%
$n = 12$	12II	
D. yina	>95%	50–70%
$n = 10$	10II	1ch3 + 9II + 1I

yina independently giving rise to *D. elegans* and *D. bacigalupii*. If *D. yina* populations were integrated through gene flow with one another, but to the exclusion of *D. elegans* and *D. bacigalupii*, *D. yina* would eventually proceed to monophyly with respect to the molecular data, and the currently recognized species would be appropriate, or could be accommodated with terms indicating their unresolved or transitional status ("metaspecies", Donoghue 1985; "ferre-species", Graybeal 1995; "plesiospecies", Olmstead 1995). What is compelling in this example is the correspondence of gene geneologies from more than one gene with geographic, cytological, and interbreeding data; a correspondence that makes a case for multiple organismal lineages (Avise 1994), and thus multiple species (de Queiroz 1998, 1999) within *D. yina*.

Geography

In the *D. yina* complex, cytological races and molecular clades appear to be roughly segregated along the Cascade Ranges (Fig. 1). When a concordant pattern emerges between phylogenetic and geographic subdivisions of a group, this often indicates little to no gene flow among subdivisions. This point has been emphasized in phylogeographic studies (Avise et al. 1987) and has received confirmation from population genetic models (Slatkin 1989). The correspondence between molecular clades within the *D. yina* complex and the distribution of these clades to either the west or east of the Cascade Ranges is striking (Figs. 1–5), and suggests that the mountain range serves as a geographic barrier to gene flow. The Cascade Ranges have been recognized as a geographic barrier in other contexts, clearly affecting differences in climate (Peck 1941; Orr and Orr 1996), and floristic composition (Peck 1941) to the west versus the east. The Klamath-Siskiyou region at the California-Oregon border is where the striking segregation of *D. yina* molecular clades to the east and west of the Cascade Ranges is much less evident (Fig. 1). Clade I is found to the west of the Cascade Ranges, except that *D. elegans* extends eastward into eastern Washington and Idaho. Clade II is localized to a region in the Cascade Range of southern Oregon (Fig. 1), in the vicinity of Lake of the Woods and Upper Klamath Lake, Oregon, and cannot readily be designated as "east" or "west". Molecular clade III is primarily east of the Cascades, but extends west into the Klamath-Siskiyou region. It is possible that the Klamath-Siskiyou region was the source from which the *D. yina* complex dispersed northward to the east and west of the Cascades, a scenario similar to hypotheses of post-glaciation dispersal presented by Whittaker (1961) and Soltis et al. (1997).

Cytology

Cytological variation within the *D. yina* complex mirrors the molecular phylogeny and the geography for the group, with $n = 12$ samples primarily east of the Cascade Ranges, and $n = 10$ samples primarily to the west. Populations of *D. yina* within Clade I have $n = 6$ or 8 in the northwestern reaches of the species range, an observation which prompted Foster (1972) to suggest a trend of decreasing chromosome numbers as one progressed from the southeast to the northwest of *D. yina*'s range. Foster's (1972) proposed explanation for this trend, based on meiotic configurations in numerous hybrids between the different chromosome races of *D. yina*, was that the races arose through Robertsonian translocations producing either a dysploid series of reductions from a starting point of $n = 12$, or a series of reductions from $n = 11$ with an increase to $n = 12$. Foster's work (1972) unfortunately did not include *D. elegans* and *D. bacigalupii*, perhaps because the potential derivation of these taxa from within *D. yina* was not reflected in the taxonomy. If *D. elegans* and *D. bacigalupii* arose from within *D. yina*, as suggested by the molecular data, the simplest explanation is that they arose from $n = 10$ and $n = 12$ populations of *D. yina*, respectively. The homology of *D. elegans* and $n = 10$ *D. yina* genomes, and of *D. bacigalupii* and $n = 12$ genomes is supported by interfertility data discussed below.

Interfertility

If *D. yina* contains the multiple divergent lineages suggested by the molecular data, one might expect levels of interfertility to correspond with the molecular clades. Indeed, levels of interfertility appear to correspond more with the molecular clades and the chromosome numbers of the populations examined than with species identification. For example, individuals of *D. yina* from Clade I show greater interfertility with *D. elegans* than with individuals of *D. yina*

from Clade III (Tables 6 and 7). Similarly, individuals of *D. yina* from Clade III show greater interfertility with *D. bacigalupii* than with individuals of *D. yina* from Clades I or II. In sum, patterns of interfertility do not appear to correspond to the species currently recognized, but do appear to correspond to chromosome races and molecular data, both of which correspond to geography.

While levels of fertility may be reduced in crosses between chromosome races or molecular clades, reproductive barriers are not complete. Nor are reproductive barriers complete among the three species currently recognized. Populations exist with hybrids between *D. bacigalupii* and *D. yina*, and between *D. elegans* and *D. yina* (Weiler 1962; Schultheis personal observation). These populations may either resemble a hybrid swarm, with a wide variety of hybrid forms, or may contain readily distinguishable parental forms and only a few hybrids (Weiler 1962; Schultheis personal observation). Regardless of whether reproductive barriers are complete or incomplete, the currently recognized species of the *D. yina* complex do not correspond to patterns of interfertility within the group.

Hypothesized Organismal Lineages Within the *Downingia yina* Complex

In sum, there appear to be three main lineages within the *D. yina* species complex. Members of the first lineage (Clade I) are characterized by either a "*D. yina*" or "*D. elegans*" morphology, and are distributed primarily west of the Cascades, with *D. elegans* extending eastward into eastern Washington and Idaho. "*D. yina*" individuals are $n = 6$, 8, or 10. "*D. elegans*" individuals are $n = 10$. Within this lineage, the "*D. elegans*" members form a clade, excluding sample *Foster 70-15-4*, from the southern periphery of the "*D.elegans*" range. The scant support for the "*D. elegans*" clade comes from morphological characters, some of which are polymorphic within populations (Table 2).

The second hypothesized lineage (Clade II), localized in the Lake of the Woods region of the Cascades in southern Oregon, is characterized by a "*D. yina*" morphology and $n = 10$. Support for this clade comes entirely from molecular characters.

Members of the third hypothesized lineage (Clade III) are characterized by either a "*D. yina*" or "*D. bacigalupii*" morphology, $n = 12$, and a distribution primarily to the east of the Cascades, into southwestern Idaho and western Nevada, and extending westward into the Klamath/Siskiyou region of southern Oregon and northern California. Within this lineage, the "*D. bacigalupii*" samples form a clade to the exclusion of sample 585-99, from the western periphery of the range. The "*D. bacigalupii*" clade is supported only by morphological characters (Table 2).

Morphological analyses presented here were unable to clearly distinguish among *D. yina* samples falling into different molecular clades (Fig. 7; Table 4), which largely correspond to variation in *D. yina* chromosome numbers. Similarly, Foster (1972) was unable to find morphological differences corresponding to the chromosome races within *D. yina*. The chromosome races and the molecular clades within *D. yina* are morphologically cryptic. Further examination of morphology may reveal differences missed thus far, but even in the absence of such differences, it is desirable to recognize what are hypothesized to be organismal lineages.

Based on the information currently available, I choose to recognize five species, with names assigned based on nomenclatural priority and the phylogenetic placement of the type specimens: *D. elegans* (Lindley) Torrey, *D. bacigalupii* Weiler, *D. yina* Applegate, *D. willamettensis* Peck, and *D. pulcherrima* Peck. Ideally taxon names, including species names, should only be assigned to clades (Mishler and Donoghue 1982; Misher and Theriot 2000). This strict application of a phylogenetic species concept only applies full species status to *D. elegans*, *D. bacigalupii*, and *D. yina sensu stricto*. *Downingia willamettensis* and *D. pulcherrima* comprise the "*D. yina*" samples from Clades I and III respectively. These samples are not resolved as clades, but may still be named as metaspecies (Donoghue 1985), plesiospecies (Olmstead 1995) or ferrespecies (Graybeal 1995). An alternative to recognizing five species is to recognize a single species, *D. elegans* (based on nomenclatural priority), and five varieties. While both alternatives recognize the same taxa, differing only in the rank applied (species or variety), the recognition of five species more clearly emphasizes the molecular, cytological and fertility diversity within this complex group. In this case, names are also available at the species rank whereas new names or combinations would be needed if the taxa were recognized at the varietal rank.

Features of the five taxa are summarized in Table 7. It is unfortunate that the three species previously referred to *D. yina* (*D. yina* s.s., *D. willamettensis*, and *D. pulcherrima*) are morphologically indistinguishable given current information. Even those features of most importance in discriminate function analysis (included in Table 7) show such overlap as to be of minimal use for field identification. Weiler (1962) did note that *D. yina* tended to be decumbent in the west and erect in the east (which would correspond to *D. willamettensis* and *D. pulcherrima* respectively), but this can be difficult to detect on herbarium sheets. This feature, as well as corolla coloration (particularly useful for distinguishing *D. elegans* and *D. bacigalupii*), is worth noting in

TABLE 7. SUMMARY OF THE FEATURES DISTINGUISHING THE FIVE SPECIES FORMERLY CLASSIFIED AS *DOWNINGIA ELEGANS*, *D. BACIGALUPII* AND *D. YINA*. * Indicates features identified in discriminant function analysis within *D. yina*. Mean ± standard deviation; based on measurements from samples in this study (Appendix 1).

	D. elegans	*D. bacigalupii*	*D. yina*	*D. willamettensis*	*D. pulcherrima*
Former classification	*D. elegans*	*D. bacigalupii*	*D. yina*	*D. yina*	*D. yina*
Cytology	$n = 10$	$n = 12$	$n = 10$	$n = 6, 8, 10$	$n = 12$
Elevation	<2000 m	<2000 m	1200–1510 m	<250 m; 650 m in Lake Co., CA	generally <2000 m
Geographic distribution	W of Cascades in Oregon and Washington; W of North Coast Ranges in California; extending eastward into eastern Washington and Idaho	E of Cascades in California and Oregon; extending westward into Klamath-Siskiyou region of northern California and southern Oregon; southwestern Idaho and western Nevada	localized to Cascades of southern Oregon, between northwestern Upper Klamath Lake and Lake of the Woods	W of Cascades in Washington and Oregon; W of North Coast Ranges in northwestern California	E of Cascades in Washington, Oregon, and California; extending westward into Klamath-Siskiyou region of northern California and southern Oregon
Morphology					
Anther angle relative to filament tube	sharply bent (84.1 degrees ± 14.4)	sharply bent (88.8 degrees ± 6.7)	not sharply bent (22.5 degrees ± 6.1)	not sharply bent (18.7 degrees ± 8.1)	not sharply bent (24.0 degrees ± 9.5)
Lower corolla lobes	nearly parallel (9.2 degrees ± 11.9)	nearly parallel (12.3 degrees ± 9.9)	divergent (46 degrees ± 12)*	divergent (59 degrees ± 12)*	divergent (52 degrees ± 12)*
Yellow in corolla throat.	absent	present	present	present	present
Filament tube	5.3 mm ± 1.5	7.5 mm ± 1.5 (longer on average than other species)	2.3 mm ± 0.5	3.0 mm ± 0.6	3.4 mm ± 1.0
Anther length	2.7 mm ± 0.5	2.9 mm ± 0.4	1.75 mm ± 0.3*	2.1 mm ± 0.3*	2.3 mm ± 0.3*
Trichomes on anther dorsal surface	generally few (can be none to abundant)	generally few (can be none to abundant)	generally less abundant than *D. willamettensis* and *D. pulcherrima* (can be none to abundant)*	generally more abundant than *D. yina* and *D. pulcherrima* (can be none to abundant)*	generally few (can be none to abundant)*

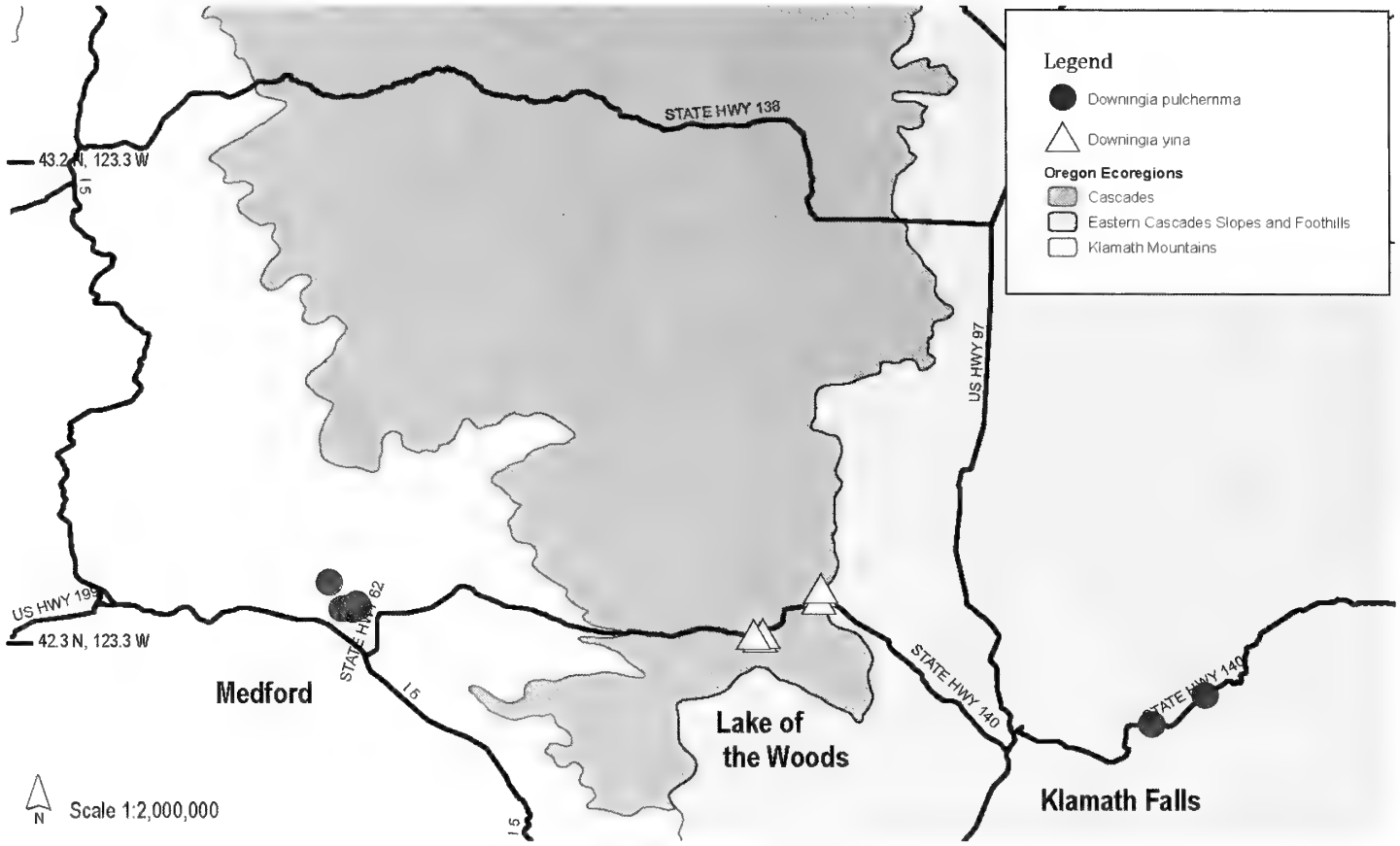

FIG. 8. Map illustrating the distribution of *Downingia yina sensu strictu* relative to adjacent *D. pulcherrima* populations in the southern Cascade Range of Oregon. The map does not illustrate *D. bacigalupii*, which is also found in the pictured region. Triangles = *D. yina* s.s. (n = 10, Clade II). Circles = *D. pulcherrima* (n = 12, Clade III). *Downingia yina* s.s. samples are located within the southern tip of the Cascades ecoregion of Oregon (following Thorson et al. 2003).

future collections. Unless reliable features are identified, we must rely on geographic location for field identification, ideally with confirmation from molecular and/or cytological data. At present I recommend that specimens collected west of the Cascades in Oregon and Washington, and west of the North Coast Ranges in California are best assigned to *D. willamettensis*. Specimens collected east of the Cascades in Oregon or Washington are best assigned to *D. pulcherrima*. *Downingia pulcherrima* is also located in the Klamath and Siskiyou regions of northern California (documented in this study as far west as Coffee Creek, just west of Clair Eagle Lake, Trinity Co.) and southern Oregon (documented in this study as far west as Medford, Jackson Co.). *Downingia pulcherrima* and *D. willamettensis* are generally above and below elevations of 250 m respectively. *Downingia yina sensu strictu* is localized to the southern tip of the Cascade Range in Oregon. This study documents populations from the northwestern edge of Upper Klamath Lake to Lake of the Woods (Klamath Co.). Based on my current understanding of the distribution for *D. yina*, I recommend assigning to this taxon any collections found in the Cascades ecoregion of southern Oregon (ecoregion as delimited in Thorson et al. 2003), while assigning those found in neighboring areas outside of this ecoregion to *D. pulcherrima*. Figure 8 provides a map delimiting the distribution of *D. yina* relative to *D. pulcherrima*.

Priorities for refining our current understanding of this species complex include obtaining molecular data from additional populations (particularly at the limits of species ranges, including Washington state) additional sampling of cytological variation, and exploration of morphological or ecological features to distinguish *D. yina sensu strictu*, *D. willamettensis*, and *D. pulcherrima*.

Key to Taxa of the *Downingia yina* Species Complex

1a. Anthers abruptly bent, >70° to filaments; lower corolla lip lobes ± parallel.
  2a. Corolla 3-colored (blue, white, yellow); lower corolla lobes obtuse, mucronate . . . . . *D. bacigalupii*
  2b. Corolla 2-colored (blue, white); lower corolla lobes acute . . . . . *D. elegans*
1b. Anthers not or ± bent, <45° to filaments; lower corolla lip lobes divergent, not parallel.
  3a. Plants generally east of Cascades, extending into Klamath Ranges in southern Oregon and northern California; generally >250 m (but <250 m along Columbia River, Washington).
    4a. Localized to southern Oregon Cascades, between northwestern Upper Klamath Lake and Lake of the Woods, plants at 1200–1510 m . . . *D. yina*
    4b. East of Cascades, extending into Klamath Ranges in southern Oregon and northern California, plants generally at <2000 m . . . . . *D. pulcherrima*

3b. Plants generally west of Cascades in Oregon and Washington, and west of North Coast Ranges in California; generally <250 m (but 650 m on Snow Mountain, Lake Co., California) . . . . . *D. willamettensis*

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APPENDIX 1

SPECIMEN VOUCHERS USED IN CURRENT STUDY, GROUPED UNDER SPECIES AND STATE. Specimens used in the morphological analysis are indicated by an asterisk. GenBank sequence accession number(s) are given for specimens used in the molecular analysis and haploid chromosome number for specimens used to collect chromosome data. Unmounted collections by Foster are deposited at DAV, accessioned collections are at JEPS or UC.

Voucher	GenBank sequence	Chromosome number
Downingia bacigalupii		
CALIFORNIA		
* *Schultheis 233-95*, Lassen Co: SW of Nubeiber, Hwy 299 at entrance to Muck Valley Hydroelectric Project (JEPS)		
* *S. Jessup s.n.*, Modoc Co.: N of Canby, 1.4 mi N of Rt 139 on Rt 46 (JEPS)		
Schultheis 230-95, Modoc Co.: N of Lookout, on Cty Rd 94, 0.3 mi S of Cedar Dr. (JEPS)	AF163376, AF163377	
* *Schultheis 231-95*, Modoc Co.: N of Lookout, at Cty Rd 94 and railroad crossing (JEPS)		
* *Schultheis 234-95*, Shasta Co.: Rt 89, 3.2 mi S of Shasta-Siskiyou county line (JEPS)		
* *Schultheis 252-95*, Sierra Co.: Sierra Valley, SW corner of road to Calpine and Rd A-23 (JEPS)		
* *Schultheis 251-95*, Sierra Co.: Sierra Valley, road A-23, 0.7 mi N of turn off to Calpine (JEPS)	AF176900, AF176878	
OREGON		
* *Schultheis 240-95*, Harney Co.: On Hwy 20, 1 mi N of junction with 395, N of Riley (JEPS)	AF163382, AF163383, AF176888	
* *Schultheis 582-99*, Jackson Co.: N of Medford, NE corner of Table Rock and Kirtland Rds. (JEPS);		
* *Schultheis 585-99*, Josephine Co.: Botanical Wayside Park at Rough and Ready Creek, S of Caves Junction (JEPS)	AF229132, AF229133, AF229151	
* *Weiler 61323*, Josephine Co.: 0.4 mi W of old site of Waldo (UC)		
* *Weiler 61319*, Josephine Co.: Hwy 199, 3.7 mi N of O'Brien (UC)		
* *Schultheis 237-95*, Klamath Co.: Rt 66, 5 mi W of Keno (JEPS)	AF229136, AF229137, AF229149	
* *Weiler 61452*, Klamath Co.: Pelican Ranger Station, 3 mi SW of Rocky Point (UC)		
Downingia elegans		
CALIFORNIA		
* *Tracy 15462*, Humboldt Co.: Larrabee Valley (UC)		
* *Weiler 60138*, Lake Co.: Snow Mountain, 3.5 mi N of Bear Creek Public Camp (UC)		$n = 10$ (Cited in Weiler 1962)
Foster 70-15-4, Lake Co.: Snow Mountain, near Kelly Cabin Site (DAV)	AF229140, AF229141, AF229152	$n = 10$ (Cited in Foster 1972 as "Snow Mt.")
* *Weiler 60256*, Mendocino Co.: S limits of Willits, E of Hwy 101 (UC)		
* *Tracy 17402*, Trinity Co.: Hettenshaw Valley (UC)		
IDAHO		
* *Aller 3000*, Benewah Co.: 1.5 mi S of Tensed (UC)		
* *Ehlers & Erlanson 39*, Bonner Co.: Edge of Lake Pend Oreille (UC)		
* *Constance 2025*, Clearwater Co.: 5 mi W of Weippe (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
OREGON		
* *Schultheis 244-95*, Benton Co: William Finley Natl. Wildlife Reserve (JEPS)		
* *Schultheis 243-95*, Benton Co.: William Finley Natl. Wildlife Reserve (JEPS)	AF163386, AF163387, AF176889	
* *Schultheis 317-96*, Linn Co.: E of Corvallis, NW corner of Hwy 34 and Looney Lane (JEPS)		
* *Schultheis 242-95*, Linn Co.: Hwy 34, 6.5 mi E of Corvallis, 1 mi E of Oakville Rd, just E of Lake Creek (JEPS)	AF163385, AF176877	
* *Schultheis 318-96*, Linn Co.: Outside of Eugene, Rt 126, 1.7 mi E of Greenhill Rd. (JEPS)		
* *Schultheis 320-96*, Washington Co.: Edge of Banks, Hwy 47, 1.6 mi N of Hwy 6, just S of Creps Rd. (JEPS)	AF163401, AF176885	
Downingia yina		
CALIFORNIA		
* *Oswald & Ahart 3943*, Butte Co.: Soda Ridge Rd, 1.4 mi W of junction W Coon Hollow Rd. (UC)	AF229146, AF229147	
* *Schultheis 247-95*, Del Norte Co.: Hiouchi. Off of Hwy 199, corner of Hiouchi and Jedediah Rds. (JEPS)	AF163388, AF163389, AF176892	
* *Schultheis 587-99*, Del Norte Co.: Hiouchi. Off of Hwy 199, corner of Hiouchi and Jedediah Rds. (JEPS)		
* *Tracy 3781*, Humboldt Co.: Alton (UC)		
* *Tracy 3217*, Humboldt Co.: Near Hydesville (UC)		
Foster 70-96-11, Humboldt Co.: Horse pasture by Hydesville School (DAV)	AF163425	$n = 10$ (Cited in Foster 1972 as "Hydesville")
* *Tracy 19527*, Humboldt Co.: Bed of former Goose Lake, in Hydesville (UC)		
* *T. O'Brien s.n.*, Siskiyou Co.: Tennant. Tennant Rd, 6.0 mi E of Hwy 97 (JEPS)	AF163439, AF176887	
* *Schultheis 580-99*, Siskiyou Co.: Trailer Lane and I-5, edge of Weed (JEPS)		
* *Schultheis 236-95*, Siskiyou Co.: Trailer Lane and I-5, edge of Weed (JEPS)	AF229134, AF229135, AF229148	
* *Weiler 60207*, Siskiyou Co.: Hwy 99, 2.3 mi NW of Weed (UC)		$n = 12$ (Cited in Weiler 1962)
* *Bacigalupi 5937*, Siskiyou Co.: 2 mi N of Weed along Hwy 99 (UC)		
Foster s.n., Siskiyou Co.: 2 mi N of Weed along Hwy 99 (DAV)		$n = 12$ (Cited in Foster 1972 as "Weed")
* *Bacigalupi 5696*, Siskiyou, CA. Hwy 99, 1.5 mi WNW of Weed (UC)		
* *Parker 100*, Siskiyou Co.: Shovel Creek, above Beswick (UC)		
Foster s.n., Siskiyou Co.: Big Flat, along Coffee Creek (DAV)		$n = 12$ (Cited in Foster 1972 as "Big Flat")
* *Douglas Barbe 348*, Trinity Co.: Trinity-Siskiyou county line, 0.5 mi N of Big Flat (JEPS)	AF229112, AF229113, AF229114	
* *Wagnon 1657*, Trinity Co.: Big Flat, along Coffee Creek (UC)		
* *Tebbe 140*, Yolo Co.; Sacramento Valley, near Woodland (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
OREGON		
* *Holmgren & Holmgren 9645*, Deschutes Co.: Hwy 20, just N of Lake Co. line (UC)		
* *Applegate 6155*, Douglas Co.: 2 mi N of Dillard, 5 mi S of Roseburg (UC)		
* *Kimber 59*, Douglas Co.: Near Drain (UC)		
* *Weiler 61332*, Douglas Co.: Hwy 99, 3 mi S of Yoncalla (UC)		
* *Weiler 61333*, Douglas Co.: 0.5 mi W of Hwy 99, 3 mi S of Yoncalla (UC)		$n = 10$ (Cited in Weiler 1962)
* *Bacigalupi 7861*, Douglas Co.: 2.5 mi S of Yoncalla, along Hwy 99 at Yoncalla-Drain exit (JEPS)		
* *Bacigalupi 7862*, Douglas Co.: 1.5 mi S of Yoncalla, just E of Hwy 236, in Pleasant Valley (JEPS)		
Foster 68-51, Douglas Co.: Off southern Yoncalla-Drain exit from I-5 (DAV)	AF163421, AF163422, AF176886	$n = 10$ (Cited in Foster 1972 as "Yoncalla")
Foster s.n., Douglas Co.: Sutherlin-Nonpariel Rd, 0.5 mi E of Platt K Road (DAV)		$n = 10$ (Cited in Foster 1972 as "Sutherlin")
* *Schultheis 241-95*, Harney Co.: Rt. 20, 2.3 mi from Lake Co. line (JEPS)	AF163384, AF176881	
* *Peck 18919*, Harney Co.: Silver Creek Valley, 10 mi W of Riley		
Foster s.n., Harney Co.: 12.5 mi S of Riley on Hwy 395 (DAV)		$n = 12$ (Cited in Foster 1972 as "Wagontire")
* *Halse 4662*, Harney Co.: Hwy 20 between Squaw and Glass Buttes, 6.2 mi E of Lake Co. line		
* *Peck 20902*, Harney Co.: 7 mi N of Wagontire (UC)		
* *Heller 15750*, Harney Co.: Hwy 395, N of Wagontire (UC)		
Robbins 4047, Jackson Co.: N of Medford (UC)		$n = 12$ (Cited in Weiler 1962)
* *Peck 24821*, Jackson Co.: Camp White (UC)		
* *Schultheis 246-95*, Jackson Co.: N of Medford, Kirtland Rd, 1.9 mi W of Table Rock Rd. (JEPS)		
* *Weiler 61195*, Jackson Co.: N of Medford, Kirtland Rd, 0.8 mi W of CampWhite-TouVelle State Park (UC)		
* *Schultheis 582-99*, Jackson Co.: N of Medford, NE corner of Table Rock and Kirtland Rds. (JEPS)		
* *Schultheis 245-95*, Jackson Co.: N of Medford, NE corner of Table Rock and Kirtland Rds. (JEPS)		
* *Weiler 61197*, Jackson Co.: Near TouVelle State Park, 0.25 mi S of Bybee Bridge (UC)		
* *Schultheis 584-99*, Jackson Co.: N of Medford, Tresham Lane just W of Table Rock Rd. (JEPS)		
Foster 70-43-15, Jackson Co.: Medford, Tresham Lane, 1 mi N of Table Rock Rd. (DAV)	AF163423	$n = 12$ (Cited in Foster 1972 as "Medford")
Foster 71-1-14, Jackson Co.: N of Medford on Hwy 66 (DAV)	AF163426, AF163427	$n = 12$ (Cited in Foster 1972 as "Agate"
* *Bacigalupi 5702*, Klamath Co.: 5 mi NE of Dairy (JEPS)		
* *T. O'brien s.n.*, Klamath Co.: Great Meadows Recreation Site, W of Klamath Falls on Hwy 140 (JEPS)		
* *Maguire 26588*, Klamath Co.: 16 mi E of Klamath Falls along Hwy 66 (UC)		
Foster s.n., Klamath Co.: 3–4 mi W of Dairy on Hwy 140 (DAV)		$n = 12$ (Cited in Foster 1972 as "Dairy")
* *Weiler 61449*, Klamath Co.: N side of Lake of the Woods (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
Weiler 61200, Klamath Co.: Lake of the Woods (UC)		$n = 10$ (Cited in Weiler 1962)
Foster 70-84, Klamath Co.: Near Lake of the Woods campground (DAV)	AF163424	$n = 10$ (Cited in Foster 1972 as "Aspen"
* *Schultheis 581-99*, Klamath Co.: W of Upper Klamath Lake, West Side Rd, 0.4 mi past O'Neil Rd N of Hwy 140 (JEPS)	AF229138, AF229139, AF229150	
* *R. Bacigalupi 7978*, Klamath Co.: Plane landing strip at NE end of Lake of the Woods (JEPS)	AF229144, AF229145, AF229153	
* *Bacigalupi 7981*, Klamath Co.: Pelican Guard Station, just W of Pelican Bay, Upper Klamath Lake (JEPS)		
Weiler 61451, Klamath Co.: Pelican Guard Station (UC)		$n = 10$ (Cited in Weiler 1962)
* *McVaugh 6303*, Klamath Co.: 3 mi SW of Rocky Point, on Lake of the Woods Rd. (UC)		
* *Cook 962*, Lane Co.: W of Eugene, 11th St where crosses flats E of Fern Ridge Lake (UC)		$n = 8$ (Cited in Weiler 1962)
Foster 71-13, Lane Co.: Road to S. J. Quam Rock Quarry off Hwy 126, W of Eugene (DAV)	AF163428	$n = 8$ (Cited in Foster 1972 as "Quarry"
Weiler 61349, Marion Co.: 0.5 mi NW of Aumsville (UC)		$n = 6$ (Cited in Weiler 1962)
* *Bacigalupi 7874*, Marion Co.: 0.5 mi NW of Aumsville (JEPS)		
* *Weiler 61354*, Marion Co.: Salem, junction of Hwy 99 and Hwy 22 (UC)		$n = 6$ (Cited in Weiler 1962)
* *Bacigalupi 7879*, Marion Co.: E of Salem, junction of Hwy 99 and OR Rd 20 (JEPS)		
* *Peck 16291*, Marion Co.: Near Aumsville (UC)		
Foster 68-210, Marion Co.: Drained marsh by Aumsville Elementary School (DAV)	AF163420	$n = 6$ (Cited in Foster 1972 as "Aumsville")
* *Schultheis 319-96*, Sherman Co.: Hwy 97, 5 mi S of Grass Valley (JEPS)	AF163400, AF176884	
* *Hitchcock 25649*, Sherman Co.: 4 mi S of Grass Valley (UC)		
* *Weiler 61383*, Wasco Co.: Western limits of The Dalles (UC)		$n = 12$ (Cited in Weiler 1962)
* *R. Bacigalupi 7894*, Wasco Co.: Western edge of The Dalles, just S of Hwy 30 (JEPS)	AF229142, AF229143	
Foster s.n., Wasco Co.: Edge of The Dalles (DAV)		$n = 12$ (Cited in Foster 1972 as "The Dalles")
WASHINGTON		
* *Sandbergh 287*, Douglas Co.: Junction of Crab and Wilson Creeks (UC)		
* *Eyerdam 1232*, Mason Co.: 20 mi SW of Shelton, on shore of small lake (UC)		
* *Meyer 1551*, Thurston Co.: 4 mi W of Olympia along Hwy 101 (UC)		
* *Sandberg & Leiberg s.n.*, Eastern WA (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
Downingia bicornuta		
CALIFORNIA		
* *Schultheis 100-95*, Tehama Co.: Dale's Lake. NE of Red Bluff, on Rt A-6 (JEPS)	AF163339, AF163340, AF176867	
Downingia concolor		
CALIFORNIA		
* *Schultheis 195-95*, Napa Co.: Junction of Pope Valley Cross Rd and Chiles-Pope Valley Rd. (JEPS)	AF163363, AF176873	
Schultheis 287-95, San Diego Co.: Grown from seed collected by E. Bauder at Cuyamaca Lake site 10 (see Bauder 1992) (JEPS)	AF163396, AF163397	
Downingia cuspidata		
CALIFORNIA		
* *Schultheis 179-95*, Calaveras Co.: SE of Camanche Reservoir, at junctions of Burson Rd, Hwy 26, and Milton Rd. (JEPS)		
Schultheis 197-95, Lake Co.: Loch Lomond. Meadow, W side of Rt 175, just N of intersection with Loch Lomond Rd. (JEPS)	AF163364, AF176890	
Downingia montana		
CALIFORNIA		
* *Schultheis 235-95*, Shasta Co.: Rt 89, 3.2 mi S of Shasta/Siskiyou county line (JEPS)	AF163378, AF163379, AF176876	
Schultheis 250-95, Butte Co.: Humboldt Rd, off of Rt 32, 0.3 mi E of Butte Meadows Natl. Forest Campground (JEPS)	AF163391, AF176894	
Downingia ornatissima		
CALIFORNIA		
* *Schultheis 180-95*, Stanislaus Co.: N of Turlock Lake. S side of Barnett Rd, off of Crabtree Rd. (JEPS)	AF163360, AF176879	

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POLLINATION AND REPRODUCTION IN NATURAL AND MITIGATION POPULATIONS OF THE MANY-STEMMED DUDLEYA, *DUDLEYA MULTICAULIS* (CRASSULACEAE)

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ABSTRACT

We investigated the reproductive biology of the rare and endangered plant, *Dudleya multicaulis* at five separate sites, three natural and two mitigation sites. We employed dawn to dusk observations to determine the spectrum of pollinators visiting *D. multicaulis*, took pollen samples from visitors to determine floral constancy, sampled nectar to determine volume produced per flower, examined the number of flowers per inflorescence, the number of those flowers that produced seed, and total seed set to determine reproductive output, completed seed germination tests to determine viability, and transplanted germinated seedlings from Petri dishes to soil to determine how well seedlings survive transplanting. *Dudleya multicaulis* was visited by flower beetles, native and European honey bees, flies, and a variety of other insects. Nectar production per flower averaged 0.12 µl. Bees averaged 99% floral constancy to *D. multicaulis*. Reproductive output measured by flower production and fruit/seed set were not significantly different among sites. Among all populations, the average fruit set ranged from 86.9 to 94.4%. The large fruit set coupled with the diversity of floral visitors suggests that *D. multicaaulis* is not pollinator limited. Data suggest that *D. multicaulis* is capable of self-pollination in absence of vectors. Seed germination and transplanted seedling survival did not differ significantly among sites. Results suggest that sowing seed may be better for plant establishment rather than transplanting when mitigation is necessitated.

Key Words: Auto-fertility, *Dudleya multicaulis*, pollination, reproductive output, seedling survival, transplanted.

Information on the reproductive biology of rare plants can provide some assistance in understanding why some plants are rare and others are common (Kearns et al. 1998). Of special importance are cases where rare plants, which are to be extirpated as a result of development, are physically transplanted to new sites or seeds from existing populations are sown in new locations intended to serve as mitigation sites. Data relative to the reproductive biology of such species should play a significant role in decision-making regarding the management, salvaging, and moving of such rare plants as part of a mitigation process. Information of this type may indeed prove critical to the success or failure of the establishment of salvaged plants or seeds in mitigation areas.

Dudleya multicaulis (Rose) Moran (Crassulaceae), the many-stemmed Dudleya, is recognized as a rare and endangered plant in California and elsewhere (List 1B.2) by the California Native Plant Society (CNPS 2005). As part of the mitigation process necessitated by the Final Project Environmental Impact Report for the Santiago Hills II Planned Community and certified by the City of Orange in 2000 (Homrighausen unpublished), this sensitive species was transplanted or seeded to new areas as part of a pilot study for future mitigation. Mitigation sites were selected based on "their similarity to the existing population sites in terms of vegetation composition and cover, apparent soil type, and depth, slope, and aspect" (Homrighausen unpublished).

A patchily-distributed geophyte, *D. multicaulis* is typically associated with the coastal sage scrub plant community of southern California (Dodero 1995; Marchant et al. 1998). Little is known about its reproductive biology (RCIP 2003), although several possible bee, fly and flower beetle pollinators are projected to be involved (Dodero 1995).

To provide information relative to the reproductive biology of this rare species, we observed the developmental sequence of flowering and investigated the pollination biology of this species during the peak flowering period in May of 2005 at the Santiago Hills site (Jones, Shropshire, and Allen unpublished), which is within the Santiago Hills II Planned Community and the East Orange development projects (Homrighausen unpublished). Subsequently, we examined the reproductive output, seed germination, and seedling survival and reproductive effort for natural and mitigation plant material. Specifically, we addressed the following questions: 1) What visits *D. multicaulis* diurnally? 2) Might the plant self without a vector? If so, what is the mechanism of

this selfing? 3) How constant are the visitors to *D. multicaulis*? 4) How much nectar is produced per flower in *D. multicaulis*? 5) What is the reproductive output in the natural and mitigation populations of *D. multicaulis*? 6) How viable are the seeds produced by plants in the natural versus the mitigation populations? 7) Do transplanted natural and mitigation population seedlings survive and reproduce during the first year?

Materials and Methods

Dudleya multicaulis is a member of the succulent family Crassulaceae (the stonecrops). Detailed descriptions of the family, genus, and this specific species can be found on line (http://ucjeps.berkeley.edu/cgi-bin/get_JM_treatment.pl?3284,3295,3324). *Dudleya multicaulis* is an herbaceous perennial that comes up each year from over wintering underground corm-like tuberous caudices. *Dudleya multicaulis* occurs on heavy clay and rocky soils in barren areas among coastal sage scrub and chaparral communities (Munz 1974) and was originally found from coastal Los Angeles County south to Camp Pendleton and inland to Riverside and San Bernardino Counties, in California.

In *D. multicaulis*, the flowering stalk is often multiple-branched and bears lemon yellow flowers. According to Munz (1974), the many-stemmed Dudleya flowers in May–June; however, both BLM (2005) and Marchant et al. (1998) give the blooming season as April–July, which is more consistent with our observations. Nascent inflorescences of *D. multicaulis* start to appear in March and April, each beginning as a pink-stemmed stalk produced near the center of the plant. Each primary stalk usually forked at least once, producing two secondary stalks. Some secondary stalks fork again, producing tertiary stalks. A single flower appears at the first fork and is the first to open. From there, blooming continues up the stalk in succession (Fig. 1). Flower "1" opens first, reaches peak bloom, if pollinated tending to develop a reddish tinge on the petals, and begins to form fruit. Relative ages of each inflorescence can be estimated by examining the condition of their flowers. Young inflorescences have their lowest flowers open and none in fruit. Intermediate-aged stalks have open flowers mid-way along the inflorescence branches with the lowest in fruit. Older inflorescences are in flower at the tips ("n" flowers) and in fruit below.

In late summer or fall, follicles dehisce and fall off of the plant. Seeds are about 0.8 mm long. Caesares and Koopowitz (unpublished) report that the average flower, with its five follicles, produces about 12 seeds, of which approximately 52% were viable when germinated under nursery conditions. All aboveground parts senesce in summer, leaving only the dried inflorescence in place. The small stature and growth habit of this species make it difficult to see and, as a result, it is easily overlooked by botanical surveyors.

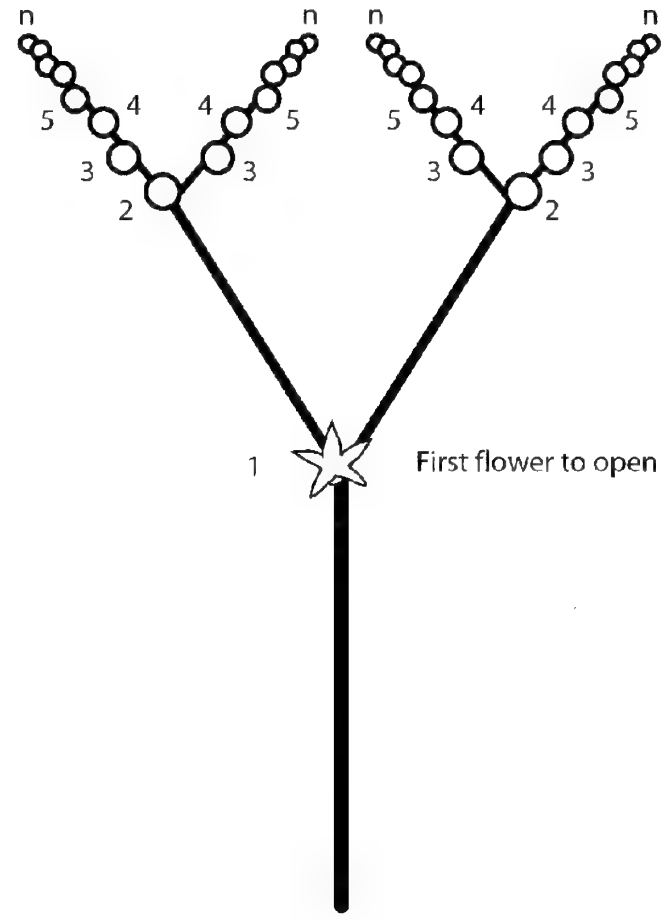

FIG. 1. Blooming sequence. Generalized flowering pattern of *Dudleya multicaulis*. 1 = first flower to open, 2 = second flowers to open, 3 = third flowers to open, 4 = fourth flowers to open, 5 = fifth flowers to open, n = last flowers to open. Flowers 6 though (n−1) were intentionally left unlabeled.

Study Sites

Primary study site. The site where the pollination studies were conducted from 13 to 15 May 2005 is located in the Santiago Hills just east, south east of Irvine Regional Park, Orange Co., California. Here a rather large population of *D. multicaulis* occurs on a northwestern facing slope near an abandoned stretch of the old Santiago Canyon Road. A series of four *D. multicaulis* subpopulations were initially delimited for study. Beginning lower on the slope and proceeding to the top of the hill, the four subpopulations were identified as follows: subpopulation C — the control site that was used for the collection of *D. multicaulis* floral visitors (insects captured at this site were used for identification and for pollen constancy studies). Subpopulations 1, 2 and 3a were dedicated for use in the dawn-to-dusk pollinator observation studies. On the second day of the dawn-to-dusk studies, subpopulation 3a was replaced by a nearby subpopulation (3b), which contained a larger number of *D. multicaulis* plants in flower. Site 3b was located at the top of the hill adjacent to the fence line separating

TABLE 1. GPS COORDINATES FOR THE SUBPOPULATIONS STUDIED AT SANTIAGO HILLS, ORANGE COUNTY, CALIFORNIA.

Subpopulation	Latitude	Longitude
C	33°47.242′N	117°44.802′W
1	33°47.240′N	117°44.792′W
2	33°47.226′N	117°44.782′W
3a	33°47.217′N	117°44.778′W
3b	33°47.215′N	117°44.772′W

the overall study site from a Toll Road (SR-261). GPS coordinates for these sites are presented in Table 1.

Ancillary study sites. The mitigation sites in Weir Canyon (GPS coordinates 33°48.784′N, 117°44.767′W) and Limestone Canyon (GPS coordinates 33°43.522′N, 117°39.721′W) were examined on 15 May 2005 and 27 May 2005, respectively, to determine how many of the mitigation plants were flowering. These plants were counted and later (on 9 July 2005 at Weir Canyon and on 14 July 2005 at Limestone Canyon) examined to determine how many of the flowers on these plants produced one or more follicles and whether these fruits contained one or more fully formed seeds. Fully formed seeds were assumed to be viable and were later utilized in the germination studies.

Pollination

Pollinators/visitors—Dawn-to-dusk observations. To determine pollinator behavior, diversity, and the relative importance of each of the major pollinator groups, a series of dawn-to-dusk surveys was conducted during the peak *D. multicaulis* bloom at the Santiago Hills study site from 13 through 15 May 2005. Peak bloom is herein defined as the time when greater than 50% of the plants were in flower. Pollinators visiting *D. multicaulis* plants were observed during at least 10 min out of each hour beginning on the hour after sun up and continuing throughout the day until 50 min after the hour before sun down. This survey involved three consecutive days of observation.

At the study site, each of the three subpopulations (1, 2, and 3) was selected on the basis of the ease with which field assistants could observe a sizeable number of plants. Two observers were employed to conduct simultaneous observations at each subpopulation during the three days of study. Each person observed and recorded the visitors to *D. multicaulis* plants and the number of flowers each visited in the initial subpopulation (e.g., 1) during the first 10 min of each hour. The observers then had 10 min to move to the second subpopulation (2) where visitors and visits were observed and recorded from 20 min after the hour until half past the hour. Finally, these same observers rotated to the third subpopulation (3) and repeated the process from 40 min after the hour until 50 min after the hour. Each day the starting subpopulation was rotated so that, during the three-day period, each of the three study plots or subpopulations was the first to be sampled at the start of the observations for that day.

A visitor was defined as any organism that actually landed on and came into contact with the anther(s) and/or the stigma(s) of the flower. Visits were defined as the number of times that a particular visitor landed on one or more flowers of *D. multicaulis* and probed that flower for nectar and/or pollen. Data were subsequently analyzed in terms of number of visitors and visits.

Pollinator/visitor collection and identification. Representative samples of visitors were collected from 13 to 15 May between 9:00 and 18:00 at subpopulation C. Organisms seen visiting three or more flowers were captured in an insect net or by using a blowing aspirator and placed in killing jars charged with ethyl acetate. Each specimen was returned to the laboratory, pinned and prepared for identification and pollen sampling. Hymenopteran samples were taken to Roy Snelling at the Natural History Museum of Los Angeles County for identification. All other visitors were identified, at least to order, by the investigators.

Pollen analysis. Each captured visitor was examined under a Bausch and Lomb dissecting microscope to determine if pollen was present on the visitor and, if so, where it was located. A 3 cm piece of double-sided Scotch® tape with one end cut to a point and that end was used to pick up any available pollen from the visitors under the dissecting scope. Once the pollen had been transferred from the visitor to the double-sided tape, the tape was placed on a 7.62 cm × 2.54 cm × 1 mm glass microscope slide. One or two drops of cotton blue (1% aniline blue in lactophenol) were added to stain the pollen grains and the slide allowed to sit for at least 24 hrs for the stain to take effect. Slides were then viewed under a Leitz compound microscope and any pollen grains present were identified as either *D. multicaulis* pollen (no other species of *Dudleya* were in flower in the local area) or foreign pollen (using pollen reference slides). The number of plant species and pollen grains found on each individual visitor was used to determine which pollinators carried the pollen of *D. multicaulis* and how constant they were to *D. multicaulis*. A minimum of 100 pollen grains were examined for each specimen, except in the case of two of the flower beetles, where only 10 and 23 total pollen grains were located and indentified. Pollinator constancy was defined on a percentage basis. The higher the percentage

of one pollen species in a sample, the more specific that pollinator was to that particular plant species. A pollinator was considered to be "constant" when that pollinator visited a given species at least 95% of the time during a single foraging flight.

Nectar samples. Near subpopulation 3b, five plants that were in bud but had no open flowers were entirely covered with light colored knee high nylon stockings on 13 May 2005. These stockings served as pollinator exclusion bags and were secured with a twist-tie to create a seal between the bag and the stem of the *D. multicaulis* plant to ensure that no pollinators visited the flowers. After approximately five days, these five plants were brought back to the laboratory where the presence of nectar was subsequently sampled using 1 µl Drummond "microcaps" disposable micro-pipettes (Drummond Scientific Company, Broomall, PA). On 18 May 2005, at least 3 newly-opened flowers on each of the five plants were probed with the micro-pipettes to determine if nectar was being produced and, if so, how much was being secreted per flower.

Reproduction

Reproductive output. Between 9 July and 14 July 2005, plants at the Santiago Hills study site, as well as the Weir Canyon and Limestone Canyon mitigation sites, were examined to determine the number of flowers produced per inflorescence and how many of those flowers contained one or more follicles. This was done to determine if there were differences in flower production per inflorescence among the sampled sites and to determine the percentage of fruit set per flowers produced. Also, while examining the fruit, mature flowers with fruit were harvested from the control (C) subpopulation at the Santiago Hills study site, from the natural population at the Weir Canyon site, and from the mitigation plants at Weir and Limestone Canyons. Two sub-samples were examined at the Weir Canyon natural population. The first sample (identified as population 1) was taken from the lower portion of the natural population on the north west facing slope and the second sample (identified as population 2) was removed from plants that co-occurred with the mitigation plants at the top of the same natural population. A total of 10 or 11 flowers were harvested at each site, one each from 10 or 11 different plants, except for the Weir and Limestone Canyon mitigation sites where more than one flower was harvested per plant to achieve a sample of 10 flowers. The number of fully formed seeds per fruit and per flower was determined.

Twenty-five inflorescences, one each from separate plants, were sampled from each of the subpopulations (C, 1, 2, 3a, and 3b) studied at the Santiago Hills site. At the Weir Canyon site, one inflorescence each from 50 naturally occurring separate plants found on the north west facing slope were examined and one inflorescence each from four of the eight plants that had been spotted and marked with flags on 15 May 2005 were examined; the other four marked plants could not be located. One inflorescence each from seven of the eight plants that had been identified and marked with flags on 21 May 2005 at the Limestone Canyon Site were also examined. The eighth flagged plant at this location could not be located. The seeds harvested from these samples were then submitted to germination tests.

Seed germination tests. A total of 208 seeds from the Santiago Hills site, 101 seeds from the Limestone Canyon site, 137 seeds from the Weir Canyon natural occurring plants, and 12 seeds from the Weir Canyon mitigation site, were harvested from the flowers produced by the plants in each of these four sites. Of these, a subsample of 100 seeds (except for the Weir Canyon mitigation site where all seeds recovered were utilized) were placed on moistened 38 lb. 8.9 cm circles of regular seed germination paper (Anchor Paper Company, St. Paul, MN) in 100 × 15 mm Fisherbrand disposable sterile petri dishes (Fisher Scientific, Los Angeles Office, Tustin, CA). A total of 18 petri dishes were utilized as follows: five petri dishes with 20 seeds per dish or 100 seeds per each were prepared for the Santiago Hills, Limestone Canyon, and the Weir Canyon natural sites. Since there were so few inflorescences produced by the mitigation planting at the Weir Canyon site, there were fewer seeds available so only 3 petri dishes with 4 seeds per each or a total of 12 seeds were prepared for the germination tests. The petri dishes were watered with 5 ml of deionized water and placed in individual Ziploc® one quart storage bags (A product of S. C. Johnson & Sons, Inc., Racine, WI), labeled with an identification code, and then randomly placed in one of two Percival Model E-30B growth chambers (Percival Scientific, Inc., Perry, IA). Each growth chamber was then set on 11 hr of daylight with 15°C daytime temperature and 10°C nighttime temperature. Germination was monitored daily from 3 October 2005 through 28 November 2005.

Transplanted seedling survival tests. A sample of the germinated seedlings from each site was transplanted into 5.5 × 5.5 × 8.5 cm (W × D × H) black plastic pots filled with potting soil on 5 January 2006 and followed through the growing season of 2006. The potting soil was a mix of an organic fraction (50%) that included peat moss (6 parts by volume) and forest humus (9 parts by volume) and of an inorganic fraction (50%) that included washed plaster sand (6 parts by volume)

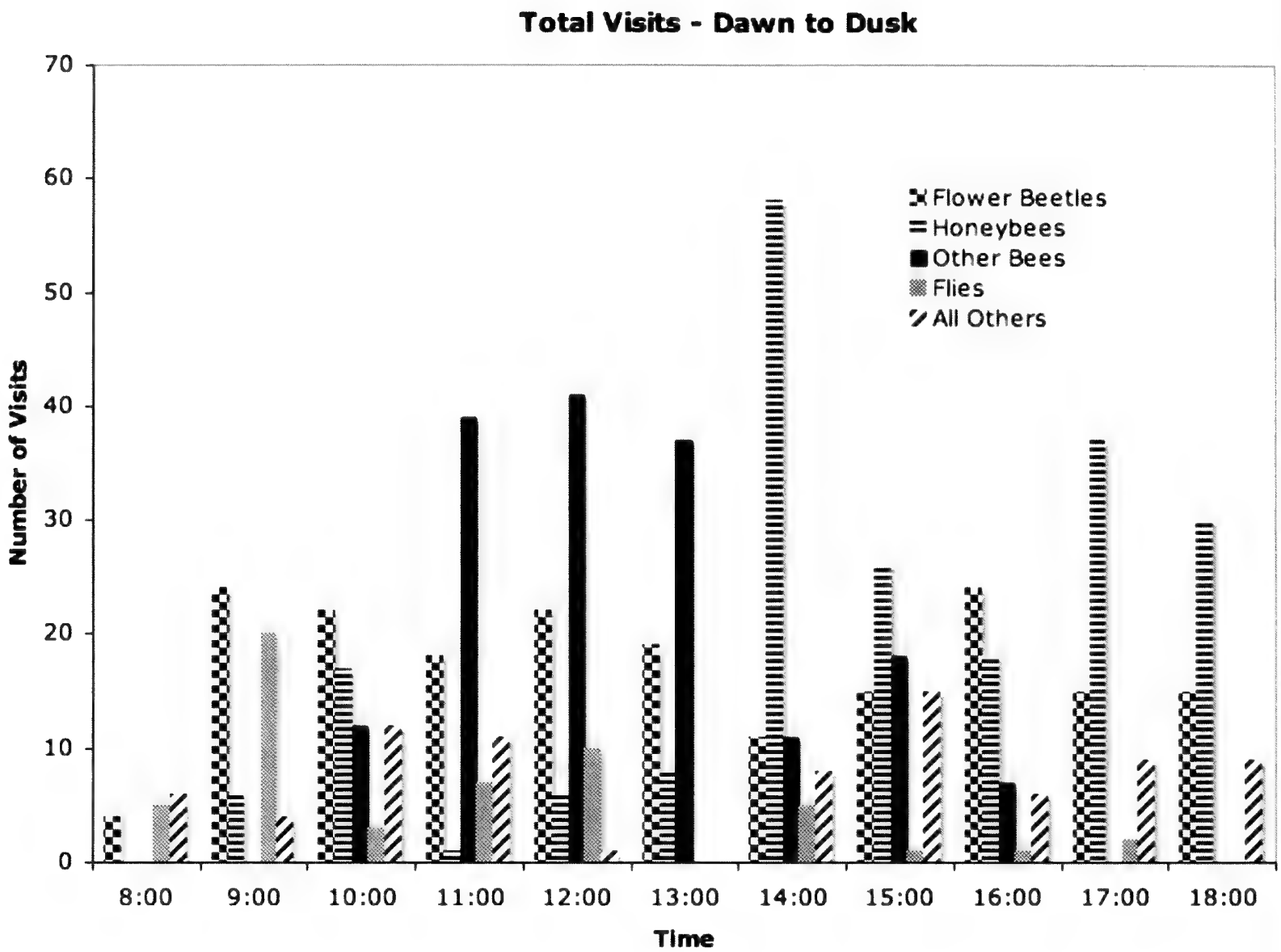

FIG. 2. Total visits by all visitors by time of day for all study plots (1, 2, 3a, and 3b) for 13–15 May 2005 combined.

and pumice (9 parts by volume). Time-released fertilizer was added at the rate of 4oz/10 gallons soil mix and dolomite (Ca and $MgCo_3$) at 5oz/10 gallons of soil mix. The time released fertilizer used was Sierrablen 18N:7P:10K + Fe.

The potted plants were placed outdoors and watered daily or as needed following rains. They were monitored for survival and reproduction at the end of the growing season on 23 June 2006.

Statistical Analyses

Sites were compared using one-way analysis of variance (ANOVA) or a Kruskal-Wallis Rank Sum Test when required for flowers produced per plant, fruit-set per flower, seeds per flower, seed germination, and survival of transplanted seedlings derived from the germination tests. Tests were done using Excel.

RESULTS

Pollination

Pollinators/visitors. Hymenopterans included the European honey bees (Apidae, *Apis mellifera* L.), two bee species in the family Halictidae (*Halictus tripartitus* Ckll. and *Lasioglossum* [*Dialictus*] sp.), one bee species in the family Megachilidae (*Hoplitis grinnelli* [Ckll]), and possibly two separate species of ants (all Order Hymenoptera). Other visitors included soft-winged flower beetles (Melyridae, Dasytinae, possibly *Lystrus* sp.) and weevils (both Order Coleoptera), several flies including those in the family Syrphidae as well as other families (Order Diptera), with only a few individuals each of true bugs (Order Hemiptera), leafhoppers (Order Homoptera), and flower mites (Order Acari). Of these visitors, the most frequent and/or most important (judging by behavior within the flowers that indicated a high probability of successful pollination) included the flower beetles, honey bees, and bees in the families Halictidae and Megachilidae.

Dawn-to-dusk observations. The results of the total dawn-to-dusk observations are summarized on a diurnal basis (Fig. 2). It is interesting to note that flower beetles were found visiting the flowers during the entire daily observational period. Non-native European honey bees tended to be more common in the afternoon hours, whereas the native solitary bees tended to frequent the flowers earlier in the day. Flies seemed to visit the

flowers early in the morning. All other visitors appeared to show a bimodal distribution arriving in the morning and then again in the afternoon.

The frequency of visits by the various groups of potential pollinators (visitors) for all study plots and all three days of observation was combined. There was considerable variation among the three subpopulations regarding the frequency of visits by the various groups, but the total frequency distribution provides a good representation of the overall visits to *Dudleya multicaulis*.

Of the total visits to *D. multicaulis*, flower beetles accounted for 31% of the visits and they represented 56% of the visitors. European honey bees and other bees, in contrast, made 27% and 19% of the visits respectively, but were represented by only 8% and 8% of all visitors, indicating that the bees were typically visiting more than one flower per foraging bout on *D. multicaulis*. Flies and all others accounted for 9% and 14% of all visits to *D. multicaulis*, but had 10% and 18% of all visitors respectively. As in the case of the visits at each of the subpopulation study sites, there was considerable variation in the relative frequency of the various groups of visitors at each study site.

Considering each subpopulation individually, 78% of the visits observed in subpopulation 1 were from flower beetles, whereas none of the other groups contributed more than 8% of the visits. Further, 84% of all *D. multicaulis* floral visitors were flower beetles.

Visits to flowers of *D. multicaulis* at subpopulation 2 were more equally distributed among the various pollinating groups with 32% of all visits being by other bees, 28% by flower beetles, and 36% by all others. Flies and European honey bees accounted for only 3% and 1% of the visits respectively. In terms of visitors, flower beetles represented 32% of the visitors, whereas other bees and all others accounted for 31% and 31% of the visitors respectively. Flies and European honey bees accounted for 3% of the visitors each.

Since we used two separate plots for subpopulation 3, two distinct patterns were observed for the visits and visitors to subpopulations 3a and 3b. Subpopulation 3a, which was utilized only on 13 May 2005, showed 44% of all visits were by the all others group, 31% by flower beetles, 17% by flies, 6% by other bees and 2% by European honey bees. An examination of the visitors for the same subpopulation shows that 40% of the visitors were in the all others group, 36% were flower beetles, 15% were flies, 6% were other bees, and 3% were European honey bees.

In contrast, visits to *D. multicaulis* flowers in subpopulation 3b, which was observed 14–15 May 2005 showed that 39% of the visits were by European honey bees, 32% by other bees, 12% by flower beetles, 11% by flies, and only 6% by all others. This represents quite a contrast with the visits observed at subpopulation 3a and may simply reflect the consequence of a much larger floral display present at subpopulation 3b in comparison to 3a. Data for visitors of the various groups at this subpopulation (3b) show that members of some of the groups made multiple visits per foraging bout (e.g., honey bees and other bees with only 14% and 25% of the visitors), whereas individuals of other groups of visitors usually visited only a single flower per foraging bout.

Pollen analysis. Pollen taken from the sampled visitors was identified. The three bee species (European honey bee, $n = 6$ and halictid bee species, $n = 5$, exhibited an average floral constancy of 98.7 and 99.7% with standard deviations of 2.61 and 0.67 respectively. The same was also true for the soft-winged flower beetle (Melyridae, $n = 4$), which had an average constancy of 74.8, but the standard deviation was much higher at 26.6.

Nectar analysis. Dudleya multicaulis plants produced an average of 0.12 µl per flower. Average nectar production per the five sampled plants varied from 0.08 µl to 0.17 µl per flower. Nectar production per flower was minimal.

Reproduction

Flower and fruit production. Data regarding the number of flowers produced per inflorescence and the percentage fruit-set for sampled plants at the various study sites/subpopulations are presented in Table 2. Although there were no significant differences among sites ($F_{8,9} = 0.94$, $P > 0.52$), the subpopulations at Santiago Hills generally produced a few more flowers per inflorescence than either of the mitigation populations at Weir Canyon or Limestone Canyon. Of the latter two, the Weir Canyon mitigation site, which was located within approximately 30 meters of a natural population of *D. multicaulis*, produced a few more flowers per inflorescence than those at Limestone Canyon, a population which was separated from a natural population of *D. multicaulis* by well over 2 km (Table 2).

There were also no significant differences for average fruit-set among sites (Kruskal-Wallis Rank Sum Test value = 4.34, $P > 0.82$). However, the average fruit-set was always greater than 85%. The range in fruit-set varied among and within the *D. multicaulis* populations from a low of 60% in the Limestone Canyon mitigation population to 100%, a high value that was found in all studied populations including the Limestone Canyon mitigation population.

TABLE 2. NUMBER OF FLOWERS PRODUCED PER INFLORESCENCE AND PERCENTAGE FRUIT-SET FOR PLANTS IN THE VARIOUS STUDY POPULATIONS AND SUBPOPULATIONS. n = the number of inflorescences sampled. Ave. fl. = average flower number, SD fl = standard deviation for that average, and R fl = range of number of flowers produced per inflorescence. Ave. % fr. = average percentage fruit set, SD fr = standard deviation for that average, and R fr = range of fruit set per flowers produced on inflorescences.

Study site/subpopulation	n	Ave. fl.	SD fl	R fl	Ave. % fr	SD fr	% R fr
Santiago Hills – subpop. C	25	12.2	5.43	6–31	91.6	7.14	78.6–100
Santiago Hills subpop. 1	25	14.6	6.33	5–33	94.4	6.20	80.0–100
Santiago Hills subpop. 2	25	13.2	5.01	6–27	86.9	9.16	64.3–100
Santiago Hills subpop. 3a	25	20.0	7.54	10–37	92.2	5.89	81.1–100
Santiago Hills subpop. 3b	25	34.5	21.42	11–94	92.5	5.99	80.0–100
Weir Canyon natural pop. 1	50	11.5	7.21	2–35	89.7	11.10	60.0–100
Weir Canyon natural pop. 2	21	11.2	7.27	2–29	94.2	7.69	71.4–100
Weir Canyon mitigation plants	4	5.3	1.71	3–7	92.3	9.0	83.3–100
Limestone Canyon mitigation plants	7	5.6	1.90	2–8	87.0	14.7	60.0–100

Seed production. No significant differences in average seed production per site was found among populations ($F_{1,6} = 3.65$, $P > 0.10$). The average seed production per flower varied by population from a low of only 0.3 in the Weir Canyon mitigation population to a high of 5.4 in the Santiago Hills subpopulation C.

The Santiago Hills population produced the highest number of fully-formed seeds per flower, (each flower having 5 separate fruits [follicles]), followed by the natural population at Weir Canyon. The mitigation plants at the Limestone Canyon site produced the next highest number of seeds per flower, whereas the Weir Canyon site produced the fewest number of seeds per flower. In fact, only one plant of the four plants sampled from this latter site contained any seeds.

Seed germination. The percentage of seeds germinating by site was not significantly different from one another (Kruskal-Wallis Rank Sum Test value = 0.17, $P > 0.98$). An examination by site showed that at least 25% of the seeds had germinated after the first 48 hr of the tests. Percent germination at each site was quite good with all sites ranging from 62% at the Limestone Canyon Mitigation Site, to 65% at the Santiago Hill Subpopulation C, to 83.3% at the Weir Canyon Mitigation Site, to a high of 85% at the Weir Canyon Natural Population. It is interesting to note that the two Weir Canyon sites had the highest germination percentages. This may be important to the ultimate survival of the population at the Weir Canyon mitigation site since so few seeds were produced by the meager number of surviving mitigation plants at that site.

Transplanted seedling survival and reproduction. Transplanted seedling survival to successful reproduction did not differ significantly by site ($F_{3,4} = 0.29$, $P > 0.83$). However, of all transplanted seedlings from all the study sites, a minimum of 25% of them survived to flowering and fruit production. The lowest survival was found in the Limestone mitigation site (at 25%) and the highest was in the Weir Canyon mitigation plants (37.5%). Conversely, between 62.5% and 75% of the transplanted seedlings died prior to maturity, indicating a relatively minimal transplantation survival rate even under the nearly ideal conditions used during this study.

DISCUSSION

Pollination by biotic agents is a mutualism that has the potential to control important aspects of plant reproduction and can play a critical role in the survival and management of rare species (Schemske et al. 1994; Kearns and Inouye 1997; Bernardello et al. 1999; Kaye 1999; Timmerman-Erskine and Boyd 1999; Spira 2001). Therefore, a knowledge of the pollination biology of any rare species takes on greater importance given the potential effect of such interactions can have on the continued existence of the rare species.

Pollinator Activity and Floral Constancy

Observations of pollinator activity were only made during the peak time of flowering. Future studies should examine pollinator activity during early, mid- and late flowering periods to determine the total spectrum of visitors (potential pollinators) and how it may or may not vary from the beginning to the end of the blooming period. The observations of pollinators within the current time frame revealed that the primary pollinators as judged by their behavior at the flowers (which included contacting the anthers and/or stigmas during a floral visit) were European honey bees and bees in the families Halictidae and Megachilidae, although flower beetles were usually the most abundant visitor at most of the plots. Six specimens of flower beetles were examined to determine if they carried *Dudleya multicaulis* pollen and this pollen of *D. multicaulis* was found on four of those individuals. Given the observed behavior of flower beetles within the flowers, the most likely role

they play in the pollination process of *D. multicaulis* is in selfing within a flower.

Our data support the suggestion that *D. multicaulis* has adopted a generalist pollination strategy (see Waser et al. 1996; Gomez and Zamora 1999, for a more detailed overview of this strategy). Plants living in fluctuating environments such as the southern California Mediterranean climate have to deal often with substantial annual variation in rainfall. Such variability in rainfall in seasonally dry environments can have a substantial effect on the number of plants that emerge from dormancy, grow to maturity, successfully flower and set fruit (Beatley 1974). The generalist pollination strategy then provides a mechanism to ensure some successful reproduction even in years in which plant population levels, flowering resources, and possibly pollinator numbers and diversity are reduced by lack of rainfall (Waser et al. 1996; Aigner 2001, 2003, 2005; Gomez and Zamora 2006). One of the potential consequences of a reduction in the diversity of potential pollinators in dry years is the potential loss of pollinator species that are more likely to effect outcrossing between plants. This occurs because flowers of species whose population levels fall low enough to reduce the floral rewards to levels that do not meet the energetic needs of the pollinators that are likely to facilitate outcrossing, such as many species of bees (Sih and Baltus 1987; Jennersen and Nilsson 1993; Conner and Neumeier 1995). As a result, selfing is more likely since remaining pollinators are ones (like flower beetles) that require fewer resources to meet their energetic needs.

Fruit Set

The total number of visitors seen visiting the flowers of *D. multicaulis* during our study was relatively small. Although fruit set varied among the subpopulations investigated, the differences were not significant. When we harvested inflorescences to determine fruit set, we found that nearly every flower had five fully developed follicles, indicating that reproduction did not seem to be pollinator limited. Fruit set was so high (in every case over 85%) that we suspected *D. multicaulis* might be at least partially self compatible (Sutherland 1986). Sutherland (1986) reviewed the fruit/flower ratios of many plant species and determined that high ratios, certainly those above 33%, were found in plants that were at least partially self-compatible. In view of the small number of visitors observed during this study and the high fruit set, we suspected that *D. multicaaulis* may not require a pollinator to effect fruit production (hence self fertile, see Harding et al. 1974; Lloyd and Schoen 1992).

Nectar Production

We found that nectar production per flower was low in comparison to species of *D.* reported for the subgenus *Dudleya* (Levin and Mulroy 1985). This reduced nectar production is a characteristic of species that do not to rely on pollinators to effect successful reproduction (Levin and Mulroy 1985).

Self Fertility

We closely examined the flowers of *D. multicaulis* and found some interesting features that may contribute to the high fruit-set in this species. Selfing without a vector within a single flower may occur. Each flower has 10 stamens, five alternate and five oppoite the petals. The five pistils begin to fold back into the groove of the V-shaped petals and their styles begin to elongate. During this process, the stigma becomes receptive to pollen deposition. If the receptive stigma does not receive pollen via normal pollinator facilitated transfer, the virgin stigma can pick up pollen as the style elongates and pushes the stigma past the anther on the stamen opposite the petal. If pollen remains on these anthers opposite the petals, selfing without a vector can occur if the pollen remains viable.

The observed floral morphology suggests that *D. multicaulis* may not require a pollinator to effect fruit production and may be able to get pollen into contact with receptive stigmas without the involvement of biotic agents. We emphasize that this is a tentative conclusion and requires further data from additional experimental procedures before it can be confirmed. Specifically, bagging or exclusion experiments are required to determine the breeding system of *D. multicaulis*. If seed is produced by selfing without a vector, then germination and seedling fitness tests should be completed. Further, any seeds produced in the bagging experiments that result from selfing with a vector (transfer of pollen from a flower on a plant to another flower on the same plant) or outcrossing should also be tested for germination and seedling survival. Levin and Mulroy (1985) found that significantly more seed was produced by outcrossing in species in the genus *Dudleya* subgenus *Dudleya* than by selfing with or without a vector and that seedlings from outcrossed seeds also survived better than those produced by either mode of selfing.

Reproductive Output

Reproductive output, as judged by seed production was not significantly different among sites and was reasonable at all sites except the mitigation plants at Weir Canyon. Of the four plants sampled from that group, only one

produced any fully formed seeds. This finding suggests that selfing does not always occur. This group of plants bears watching and may not survive with such low reproduction. Average seed production per flower also did not vary significantly among our study populations (ranging from a low of 0.3 to a high of 5.4 seeds per flower) and were much lower than the approximately 12 seeds produced per flower found by Casares and Koopowitz (unpublished).

Seed Germination and Seedling Transplantation

Tests were completed on the seeds produced by *D. multicaulis* to see if they will germinate and result in successful offspring. The seed extracted from plants from each of the four study sites germinated quite well and the percent germination was not significantly different among sites. Germination ranged from 62% for the Limestone Canyon mitigation site to 85% for the Weir Canyon natural site. Our germination results are higher than those found by Caesares and Koopowitz (unpublished) who recorded a germination rate of about 52% under nursery conditions. It would appear that seeds produced by all plants demonstrate sufficient viability to ensure successful seed reproduction. Further, when the germinated seedlings from these seeds were transplanted into pots and placed out-of-doors under relatively normal conditions, except for regular watering, between 25% (Limestone Canyon mitigation site) and 37.5% of the plants (Weir Canyon mitigation site) survived and successfully produced one or more seeds by the end of the first year. It should be noted, however, that between 62.5% and 75% of all transplanted seedlings died during this first year when they were grown under nearly ideal conditions. Transplanting of seedlings or adult plants to new locations would not seem to be a viable alternative to sowing harvested seed as a mitigation measure for this species. It should be noted that the two mitigation sites were dissimilar in that the Weir Canyon mitigation site was within approximately 30 m of an existing natural population, whereas the Limestone Canyon mitigation site was quite remote from any existing natural population of *D. multicaulis* (ca. 2 km).

Reproductive Strategies

Wilken (unpublished) investigated the reproductive strategies of *D. nesiotica* (Moran) Moran, another member of the subgenus *Hasseanthus* and concluded that it is self-compatible but requires a vector to facilitate reproduction. Levin and Mulroy (1985) studied the pollination biology of several species in the genus *Dudleya* subgenus *Dudleya* and found that two of the three major groups of species in this subgenus demonstrated a significant degree of self-fertility. They attributed this to unreliable pollinators and/or environmental unpredictability. By unreliable pollinators, they meant pollinators that varied considerably in abundance both temporarily and spatially (Levin and Mulroy (1985). In our study, pollinator abundance appeared to be minimal. It could be that the past few drought years have had a negative effect on insect populations. It may take a few wet years for insect populations to return to normal.

Environmental variability, and thus unpredictability of resources and pollinators, has certainly been a factor in the development of southern California ecosystems as rainfall varies considerably in both amount and pattern from year to year. Therefore, if self-fertility is found to be a significant mode of reproduction in *D. multicaulis*, then it may represent an adaptation that increases overall reproductive success in habitats like the coastal sage scrub community and for species like *D. multicaulis* (Moeller 2006). However, it again needs to be emphasized that in *Dudleya* subgenus *Dudleya*, selfing with a vector and outcrossing both resulted in more seed production and, in the case of outcrossed seed, better fitness of the seedlings (Levin and Mulroy 1985). Similar seed set results were also found for *D. nesiotica* in that it produced about the same fruit set when manually selfed (22.1 seeds per flower) or when outcrossed (20.3 seeds per flower). However, if emasculated and unpollinated, no fruit set occurred (Wilken unpublished). Wilken (unpublished) provides no data relative to the possibility that self-fertility can occur within flowers in time, if vector facilitated pollination does not occur before the senescence of the flower.

Self pollination is also prevalent in habitats with short growing seasons (Runions and Geber 2000; Mazer et al. 2004). *D. multicaulis* occupies such a habitat, one characterized by extreme annual variation in rainfall, which tends to favor small flowers (Strauss and Whittall 2006). Smaller flowers like those found in *D. multicaulis* increase the likelihood of selfing because of the close proximity of the anthers and stigmas (Snell and Aarssen 2005). This association of small flower size and variable water availability has been shown to increase selfing in several annual plants genera (Guerrant 1989).

The breeding biology of a rare species is a very important issue that requires careful consideration by decision makers when movement of plants is required for mitigation purposes. For example, if a rare plant requires no pollinator and still sets abundant seed, and assuming such seed germinates and the progeny survive, then, at least in the short term the sowing of this seed may increase the probability of successful mitigation

in cases where plants must be removed from a site. This appears to be the best option for *D. multicaulis*.

However, selfing can have more long-term consequences that include increased inbreeding depression and increased homozygosity in the interbreeding population and, thus, decreased genetic variation at the colony scale. One of the goals of many conservation programs is to maintain genetic diversity in species that are rare, threatened, or have small population size like *Dudleya multicaulis* (Frankel and Soulé 1981; Simberloff 1988). For this reason, genetic studies of rare plants should be completed whenever possible. Information from these studies can establish much about the species that will assist in its successful management (Ellstrand and Elam 1993).

Genetic Structure and Selfing

In a previous study of the genetic structure of *D. multicaulis* by Marchant et al. (1998), they concluded that there is little evidence for significant gene flow between populations and that local populations tended to show heterozygote deficit. They also indicated that reduced genetic variability within populations of *D. multicaulis* might be a consequence of founder effects and subsequent mating among relatives. We would add that selfing should also be considered. In this regard, Marchant et al. (1998) did note that *D. multicaulis* can self, but indicated that they had not investigated if selfing in *D. multicaulis* lowered the fitness of the progeny. Data from Levin and Mulroy's study (1985) of *Dudleya* subgenus *Dudleya* suggest that lowered fitness may indeed be the case.

Marchant et al. (1998) additionally state that variation among *D. multicaulis* populations tended to be significant, further indicating that gene flow by either pollen transport or seed dispersal was limited. How far apart, then, must *D. multicaulis* populations be for genetic isolation to be significant? That remains to be determined for *D. multicaulis*, but an interesting recent study by Boose et al. (2005), examined genetic variation in *Navarretia leucocephala*, and concluded that distances of 1100 to 1800 m were often sufficient to result in significant genetic differentiation between populations. Therefore, for species like *D. multicaulis* with limited pollen and seed dispersal capabilities, it is quite probable that significant interpopulational variation in genetic structure could occur at these distances or less.

However, it may be that selfing is a key component in the survival of this species. A recent paper by Morgan et al. (2005) demonstrated, using models, that plants with population densities that vary annually with environmental conditions (like *D. multicaulis*) may avoid extinction by increased reliance on autogamy, especially when they are pollinated by generalist pollinators (as is the case with *D. multicaulis*). Further, their models also showed that delayed selfing is always favored. At least selfing without a vector appears to occur in *D. multicaulis* only if pollinator services are not forthcoming since *D. multicaulis* is protandrous. If our model of selfing without a vector is shown to be a functional mode of reproduction in *D. multicaulis*, it may mean that newly established mitigation populations may be able to persist without going extinct because of their ability to self without a pollination vector. In fact, they may be able to persist long enough to develop sufficiently large plant populations to attract the generalist pollinators required to facilitate outcrossing and increase genetic diversity (Jarne and Charlesworth 1993).

Conclusions

There is much more research to be done to elucidate the reproductive biology of *Dudleya multicaulis* to provide the background data required to increase the probability of the successful preservation of this species. However, we suggest that transplantation of plants to new sites may not be as good a mitigation measure as seeding the new sites with seeds derived from those plants. It should be noted that our germination tests were completed under controlled conditions suggesting that artificial watering following seed inoculation of a new location may be necessary to ensure adequate germination and survival.

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A NEW SPECIES OF *DISTICHLIS* (POACEAE, CHLORIDOIDEAE) FROM BAJA CALIFORNIA, MEXICO

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ABSTRACT

Based upon a specimen first collected by S. N. Stephenson, a new grass species, **Distichlis bajaensis** H. L. Bell, is described. Stephenson hypothesized that this specimen was a hybrid between *D. littoralis* and *D. spicata*. Analyses of sequences of nuclear internal transcribed spacer (ITS) and chloroplast *ndhF* and *trnL–trnF* and an examination of gross morphology, blade and lemma micromorphology, and blade transectional anatomy demonstrate that this grass is a new species that may be sister to the remaining *Distichlis*. The blades of **D. bajaensis** are yellow-green; those of *D. littoralis* and *D. spicata* are blue-green. **Distichlis bajaensis** can be distinguished from *D. littoralis* by its exserted inflorescences with glumes present and from *D. spicata* by its short (0.8–1.5 cm) blades with a bend toward the adaxial side. At and distal to the bend, there are antrorse hairs along the medial vascular bundle. **Distichlis bajaensis** is known from a single large population growing along alkaline seeps in Arroyo Rosarito in Baja California, Mexico.

RESUMEN

Se describe como especie nueva de las gramíneas a **Distichlis bajaensis** H.L. Bell, basada en un espécimen colectado por la primera vez por S. N. Stephenson. Stephenson postuló que este espécimen era un híbrido de *D. littoralis* y *D. spicata*. Los análisis de secuencias de ADN nuclear (ITS) y del cloroplasto (*ndhF* y *trnL-trnF*), así como los estudios de morfología general, micromorfología (lema y lámina) y anatomía (hoja), demuestran que esta gramínea es una especie nueva y que puede ser hermana a las especies restantes de *Distichlis*. Las hojas de **D. bajanensis** son amarillentos verdes pero estas de *D. littoralis* and *D. spicata* son azulinos verdes. Es posible diferenciar **D. bajaensis** de *D. littoralis* por las inflorescencias exsertas con glumas y de *D. spicata* por las hojas cortas (0.8–1.5 cm) con una curva hacia la cara abaxial. Hay pelos antrorsos a lo largo del nervio central antes del medio. **Distichlis bajaensis** se conoce de una sola población grande que crece a lo largo de filtrars alcalinas en la localidad de Arroyo Rosarito, Baja California, México.

Key Words: Baja California, Chloridoideae, *Distichlis*, halophytic grass.

A putative hybrid between *Monanthochloë littoralis* Engelm. and *Distichlis spicata* (L.) Greene from Baja California, Mexico was reported by Stephenson (1971). The putative hybrid resembled *M. littoralis* in vegetative morphology and *D. spicata* in inflorescence structure. *Monanthochloë littoralis* is distributed in coastal regions of subtropical Mexico and USA with one inland population known from Coahuila, Mexico. *Distichlis spicata* has a much broader distribution in coastal and inland North and South America.

Recent work has placed *Monanthochloë* into synonymy with *Distichlis* based upon anatomical, morphological, and molecular evidence (Bell and Columbus 2008). Thus, *M. littorialis* is hereafter referred to as *D. littoralis* and the putative hybrid is considered interspecific. The present study was undertaken to determine if the population from Stephenson (1971) was still extant and to test whether the plants belonging to this population are hybrids, as Stephenson hypothesized.

A hypothesis of hybrid origin predicts that incongruence may be observed between phylogenies derived from nuclear (biparentally inherited) and chloroplast (uniparentally inherited) DNA sequences (McDade 1992; Rieseberg et al. 1996 and refs. therein; Blattner 2004; Jakob and Blattner 2006). To test this hypothesis, I present new sequence data derived from both nuclear and chloroplast genomes. These data are added to existing matrices from Bell (2007) and Bell and Columbus (2008). In addition, whole plant morphology, micromorphology of the abaxial surfaces of blades and lemmas, and blade transectional anatomy of the putative hybrid plants were studied and compared to other species of *Distichlis*.

METHODS

During the springs of 2008 and 2009, extensive searches for historical populations of the putative hybrid were conducted near El Nuevo Rosarito in Baja California, Mexico. Observations were made of the growth habit and site conditions. Leaf material was dried in silica gel for DNA analysis, blades, culms, and spikelets were

preserved in FPA (formalin:propionic acid:ethanol, 1:1:18) for anatomical investigations, and pressed, dried herbarium specimens were prepared for morphological studies. For the remainder of this report, the putative hybrid will be referred to as Baja grass.

Genomic DNA was extracted from leaf tissue from *Stephenson 68-304a* (MSC 216526) and freshly collected material (*Bell 458*, RSA 754084) using DNeasy Plant Mini Kits (Qiagen, Valencia, CA). Sequences of the nuclear ribosomal internal transcribed spacer (ITS) as well as chloroplast *ndhF* and *trnL–trnF* were amplified using primers and protocols described in Bell and Columbus (2008). In order to detect possible allelic variation in ITS, the amplification product was cloned using a TOPO TA kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Ten colonies per sample were screened. Cycle sequencing was conducted at Rancho Santa Ana Botanic Garden on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) following the protocols of Bell and Columbus (2008).

Sequences were assembled, edited, and incorporated into existing alignments from Bell (2007) and Bell and Columbus (2008). The same outgroup taxa used in Bell and Columbus (2008) (*Allolepis texana* (Vasey) Soderstr. & H. F. Decker; *Bouteloua dactyloides* (Nutt.) Columbus; *Eragrostis obtusiflora* (E. Fourn.) Scribn.; *Jouvea pilosa* (J. Presl) Scribn.) were employed in this study. Maximum parsimony (MP) and Bayesian inference (BI) analyses were conducted on the ITS and combined chloroplast (*ndhF* + *trnL–trnF*) data sets using PAUP* (Swofford 2002) and MrBayes vers. 3.0b4 (Huelsenbeck and Ronquist 2001) using the search parameters outlined in Bell and Columbus (2008). In addition, ITS 1 and ITS 2 were analyzed separately (Yokota et al. 1989; Liu and Schardl 1994; Mai and Coleman 1997). Branch support was assessed via posterior probabilities (PP), parsimony bootstrap (BS), and Bremer Support Values (BSV) (Bremer 1988) following Bell and Columbus (2008). Sequences generated during this study were submitted to GenBank and accession numbers are given in Appendix 1.

Abaxial surfaces of leaf blades and lemmas were observed following the methods of Bell and Columbus (2008). Transectional anatomy of leaf blades was examined following Columbus (1999). Descriptive terminology follows Ellis (1976) for anatomy and Ellis (1979) for morphology.

Results

Collection Site

As described by Stephenson (1971), I found Baja grass growing along alkaline seeps in Arroyo Rosarito adjacent to Mexico Hwy 1, southwest of El Nuevo Rosarito, approximately 100 km north of the border with Baja California Sur. Coordinates of the collection site are 28°43′36″N 114°43′17″W.

Baja grass was one of the dominant species at the site and one of the few grasses present although some *D. spicata* was noted also. No *D. littoralis* was observed. Stephenson found fragments of both male and female plants at the heavily grazed site in 1968; only male plants were located during my extensive searches. Burros and cattle were observed in the area, but the population was not heavily grazed during the time of my collections in 2008 and 2009. Morphological features of the earlier collection (*Stephenson 68-304a*, MSC 216526), e.g., exserted inflorescences, spikelets with glumes, suggested affinities to *D. spicata*. However, in the field, its growth habit resembles that of *D. littoralis*; thus, it is clear why Stephenson would have considered *D. littoralis* to be a possible parent. Like *D. littoralis*, Baja grass possesses stolons and frequently grows up through (as on a trellis) adjacent plants such as species of *Juncus* and *Lycium*. The leaves of both *D. littoralis* and *D. spicata* are usually dark blue-green; those of Baja grass are yellowish-green.

DNA Sequence Analysis

Nine of ten cloned ITS sequences from Baja grass (*Stephenson 68-304a*) were identical; the tenth sequence differed by a single base pair. Sequences from recently collected material (*Bell 458*) of ITS (to the group of nine) and *ndhF* were identical to those generated from *Stephenson 68-304a*; only sequences from *Stephenson 68-304a* (including *trnL–trnF*) were used in the analyses. Descriptive statistics for the MP analyses are given in Table 1. Both specimens of Baja grass (*Stephenson 68-304a* and *Bell 458*) showed a single unique indel, a three base pair repeat in ITS.

The trees with the highest log-likelihood value are shown in Figure 1 (ITS) and Figure 2 (combined chloroplast). In the ITS analyses, Baja grass is supported as sister to all other *Distichlis* (BS = 91%, PP = 1.00, BSV = 4). In the combined chloroplast tree, Baja grass is retrieved in a polytomy with all other *Distichlis* species with good support for the clade (BS = 99%, PP = 1.00, BSV = 7). When ITS 1 and 2 were analyzed separately, the topological position of Baja grass changes (data not shown). With ITS 1 (in the MP strict consensus tree), Baja grass resolves as sister to *D. laxiflora* and *D. scoparia*; with ITS 2, it resolves as sister to the *D. spicata* clade. However, neither of these positions was supported. In addition, when an ITS sequence from the Baja grass was aligned and analyzed with a dataset

TABLE 1. DESCRIPTIVE STATISTICS FOR MAXIMUM PARSIMONY ANALYSES. MP = maximum parsimony, PIC = parsimony informative characters, CI = consistency index, RI = retention index.

Region	Aligned length	% missing data	# of MP trees	MP tree length	PIC	CI	RI
ITS	641	0	26	500	148	0.76	0.82
ndhF + trnL–trnF	2111 + 1040	0.1	209	275	84	0.83	0.86

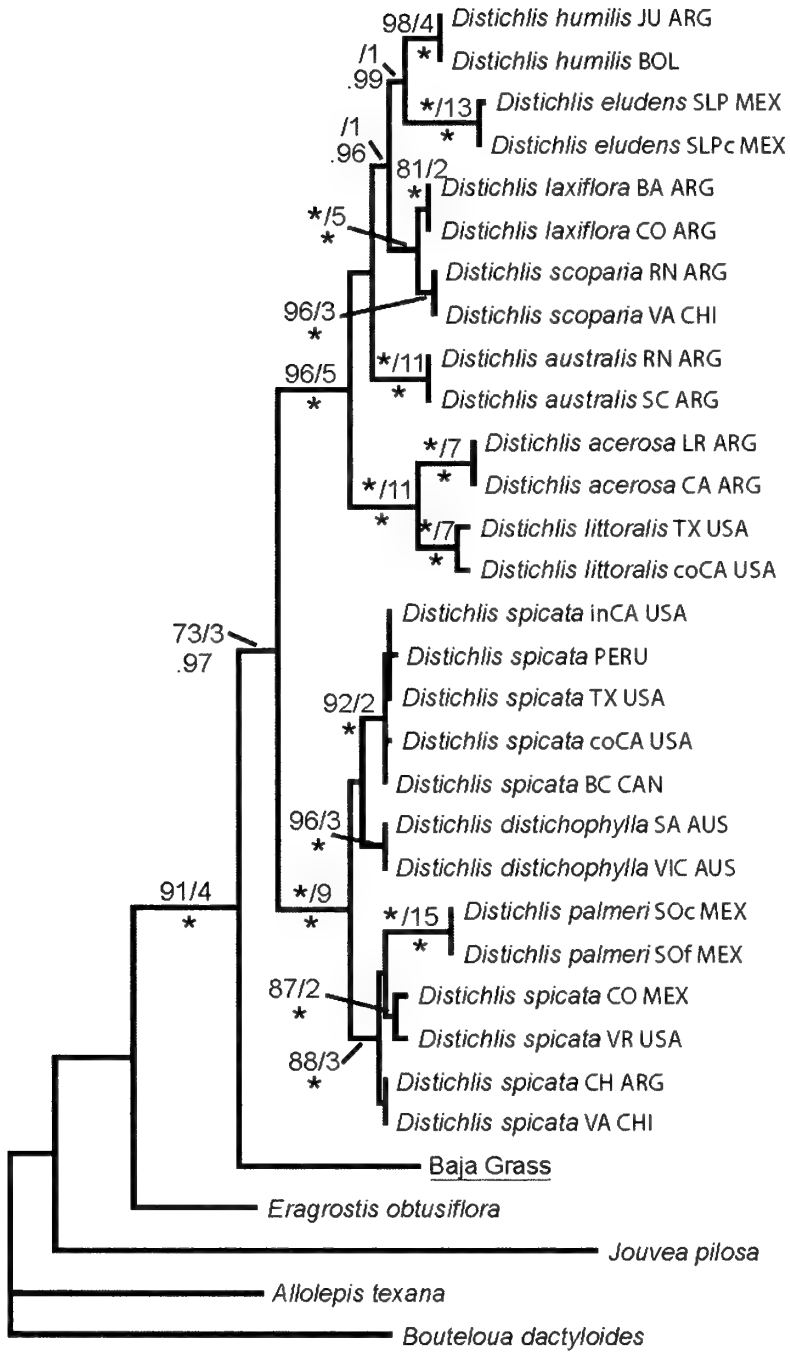

FIG. 1. Tree with the highest log-likelihood score from Bayesian analysis of ITS. Bootstrap values followed by Bremer support are given above the branches, and below are posterior probabilities. An asterisk indicates 100% bootstrap or 1.00 posterior probability. Branches marked with an arrow collapse in the strict consensus from parsimony analysis. Geographical abbreviations are as follows: ARG = Argentina (BA = Buenos Aires, CA = Catamarca, CH = Chubut, CO = Córdoba, JU = Jujuy, LR = La Rioja, RN = Rio Negro, SC = Santa Cruz); AUS = Australia (SA = South Australia, VI = Victoria); BOL = Bolivia; BC CAN = British Columbia, Canada; CHI = Chile (AN = Antofagasta, VA = Valparaiso); MEX = Mexico (CO = Coahuila, SLP = San Luis Potosí, SO = Sonora); USA (CA = California, TX = Texas, VR = Virginia).

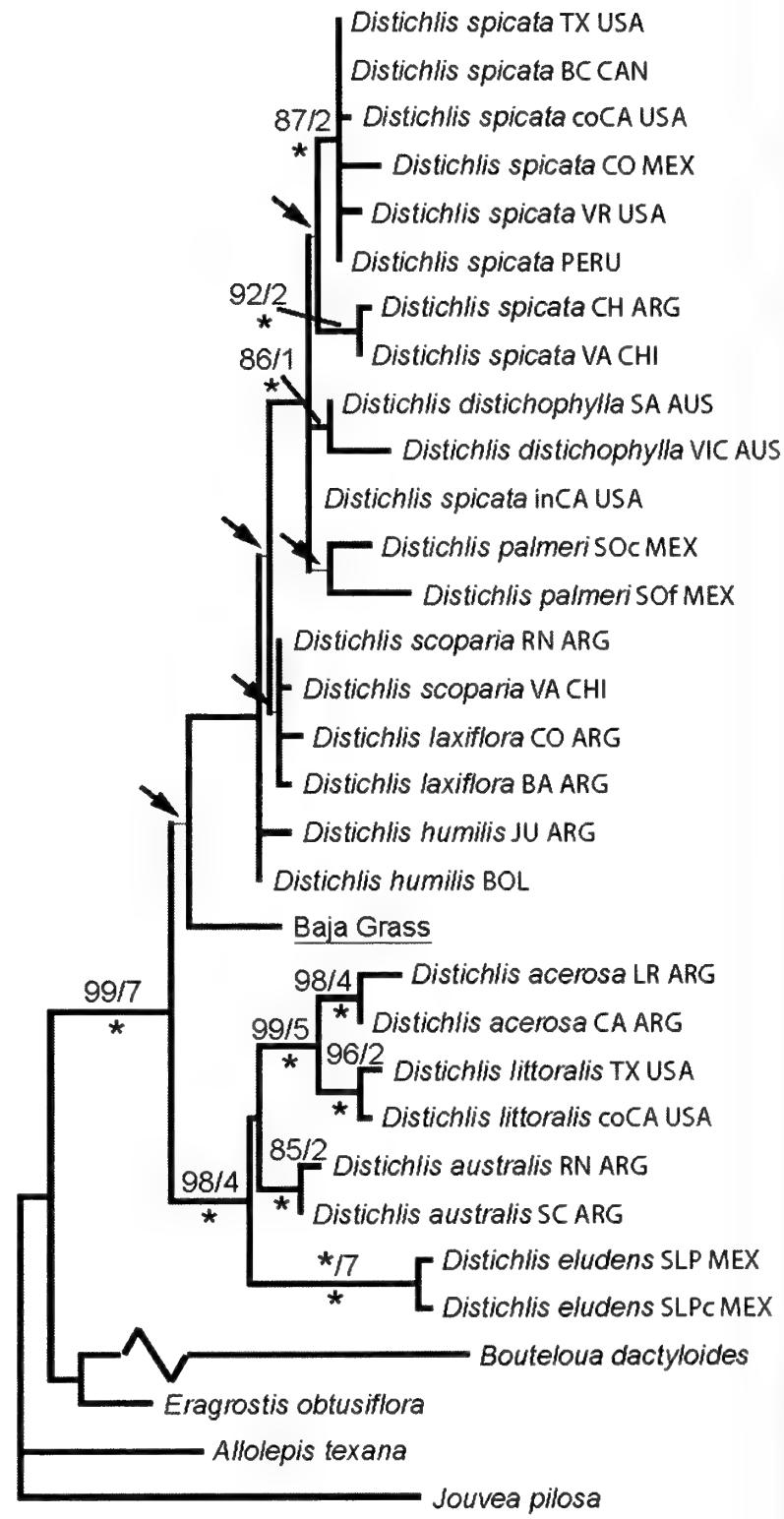

FIG. 2. Tree with the highest log-likelihood score from Bayesian analysis of combined chloroplast data set (*ndhF + trnL–trnF*). Bootstrap values followed by Bremer support are given above the branches, and below are posterior probabilities. An asterisk indicates 100% bootstrap or 1.00 posterior probability. Branches marked with an arrow collapse in the strict consensus from parsimony analysis. Geographical abbreviations are the same as in Fig. 1.

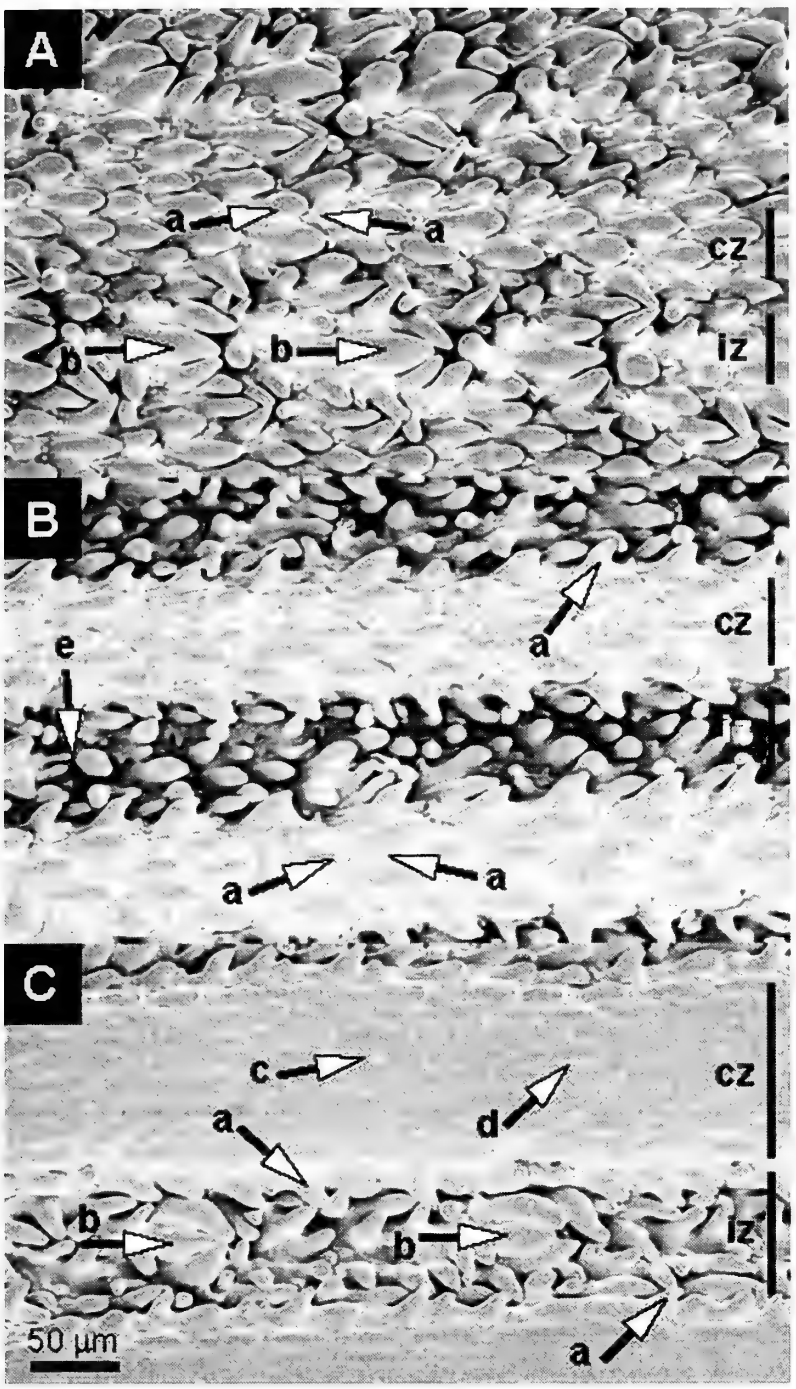

FIG. 3. Comparisons of abaxial blade surfaces; A. Baja grass (*Bell 458*), B. *Distichlis spicata* (*Bell 231*), C. *Distichlis littoralis* (*Bell 260*). a = papilla, b = clustered papillae, c = short cell, d = long cell, e = microhair, cz = costal zone, iz = intercostal zone. Scale bar applies to A, B, and C.

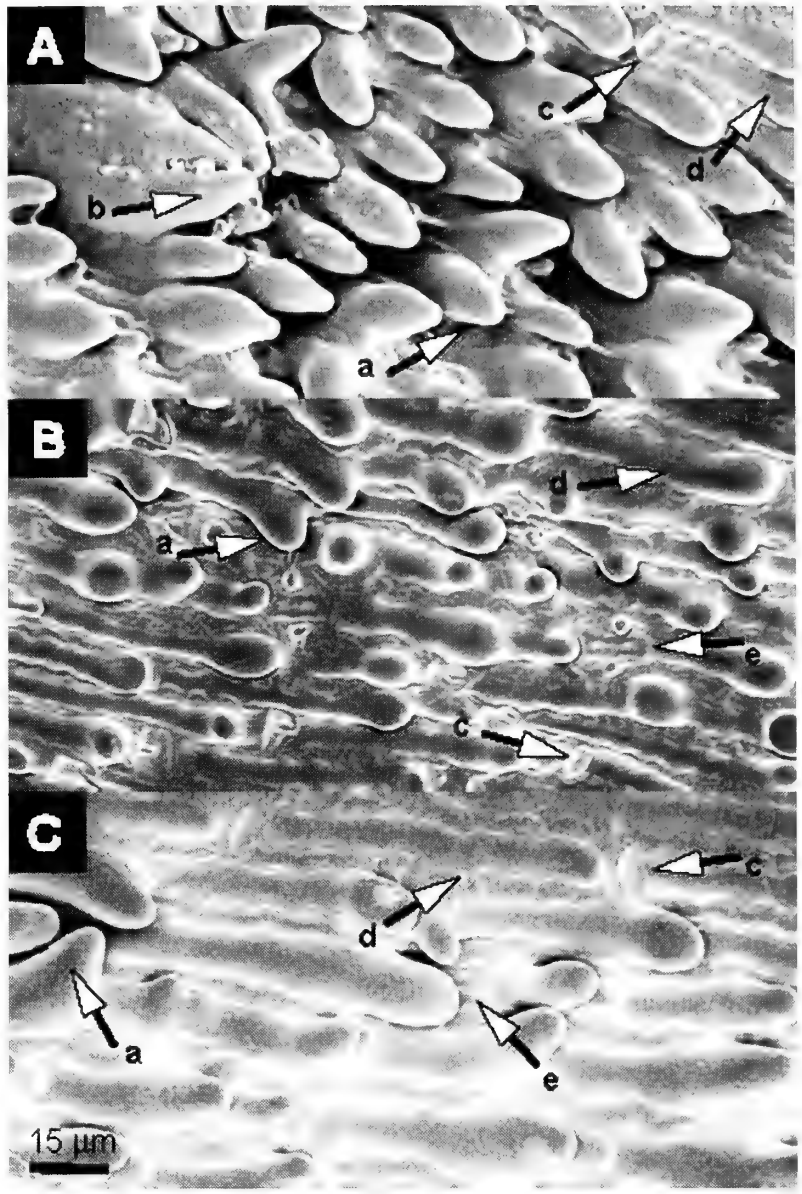

FIG. 4. Comparisons of abaxial surfaces of lemmas; A. Baja grass (*Bell 458*), B. *Distichlis spicata* (*Bell 277*), C. *Distichlis littoralis* (*Bell 260*). a = papilla, b = clustered papillae, c = short cell, d = long cell, e = stomate. Scale bar applies to A, B, and C.

derived from 84 chloridoid genera (Bell 2007), it resolved as sister to *Distichlis* (data not shown).

Micromorphology

Abaxial surfaces of leaf blades of Baja grass are highly papillate making it difficult to observe features such as long and short cells, microhairs and stomates (Fig. 3A). In the costal zones of blades of Baja grass and *D. spicata*, there are regular pairs of large and small papillae (Fig. 3A, B). In intercostal zones of Baja grass and *D. littoralis*, papillae form complexes associated with microhairs (Fig. 3A, C). In Baja grass and species of *Distichlis*, stomates occur in two files along each edge of the intercostal zone; stomates are frequently obscured by complexes of papillae making them difficult to observe from a surface view. Stomates of Baja grass and *Distichlis* have dome shaped subsidiary cells.

Abaxial surfaces of lemmas of Baja grass have many papillae that obscure features such as microhairs and stomates (Fig. 4). There are many complexes of papillae similar to those found on species of *Distichlis*. Microhairs and stomates appeared to be more sparse on lemmas of Baja grass than in *Distichlis* but they may be hidden by papillae.

Anatomy

Blade transectional anatomy of Baja grass is similar to that of *Distichlis* species (Fig. 5). The outline of the blade transection is broadly U-shaped. There are adaxial furrows between all vascular bundles to a depth of about half of the blade thickness. Furrows are absent or shallow on the abaxial side. Blades possessed about 14 total vascular bundles, three of which were 1st order. Examination of species of *Distichlis* found from 18–24 (7–9 1st order) vascular bundles in blades of *D. spicata* and 9 (3 1st order) in *D.*

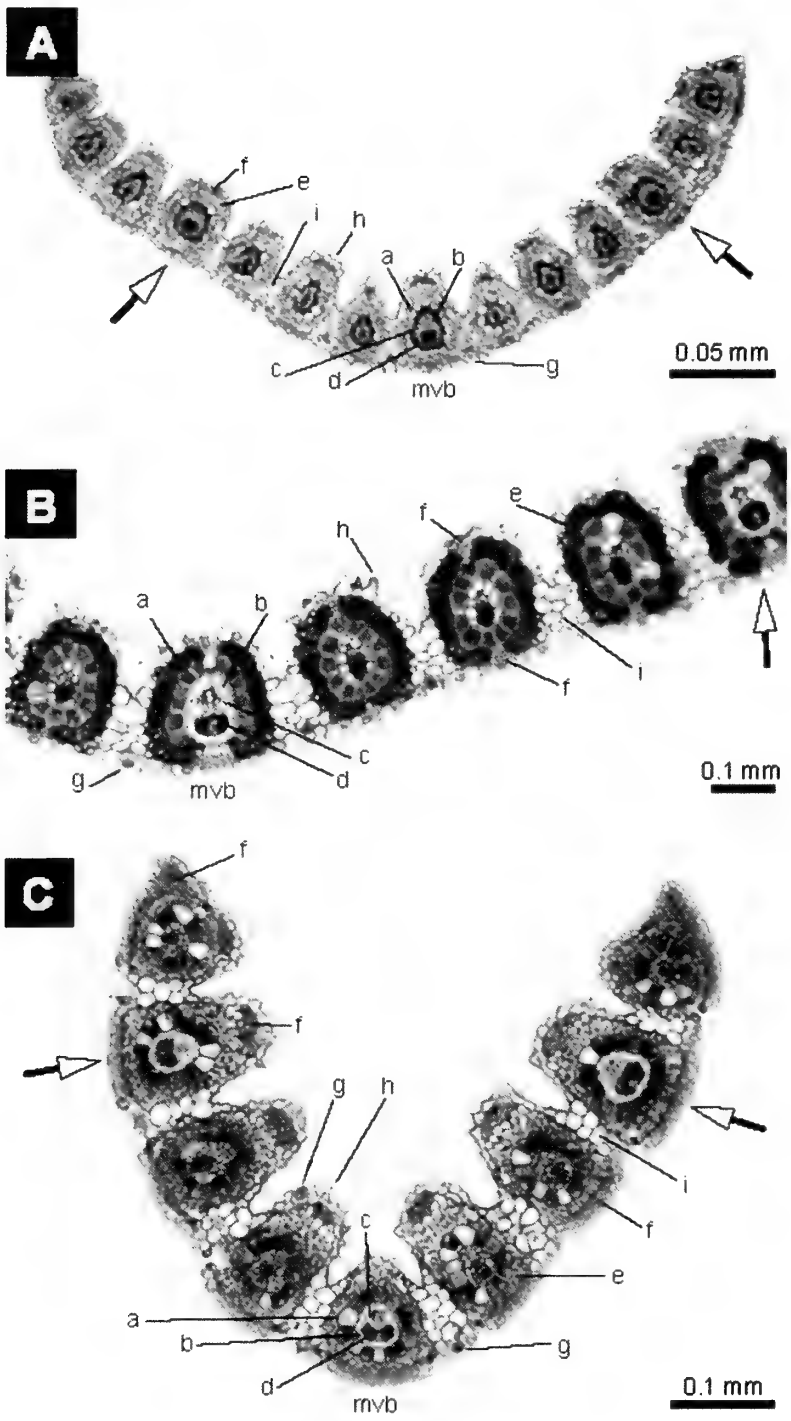

FIG. 5. Comparison of blade anatomy; A. Baja grass (*Bell 458*), B. *Distichlis spicata* (*Bell 375*), C. *Distichlis littoralis* (*Bell 260*). a = outer bundle sheath, b = inner bundle sheath, c = xylem, d = phloem, e = mesophyll, f = sclerenchyma, g = microhair, h = papillae, i = colorless cells.

littoralis (Bell and Columbus 2008). Second order vascular bundles form a regular arrangement between the 1st order bundles; a single 3rd order bundle is found at each margin. Sheath cells in all vascular bundles are elliptical in shape. The outline of 3rd order bundles is round and that of 1st and 2nd order bundles are elliptical. First order vascular bundles have a continuous double sheath that is not interrupted and lacks extensions. Phloem is directly adjacent to the inner sheath, and metaxylem is narrow. Walls of the inner sheath are thickened. Chloroplasts are centripetally arranged in the outer sheath cells. Very narrow strands of sclerenchyma are found on both adaxial and abaxial sides of most vascular bundles, and a small sclerenchyma cap occurs at the margins. Mesophyll forms a single layer of radially arranged cells. Colorless cells form uni- to multiseriate columns between all vascular bundles. Bulliform cells are associated with colorless cells at the base of furrows. Other epidermal cells are small and have numerous papillae on both surfaces. First order vascular bundles of Baja grass show Kranz anatomy of the type that predicts NAD-ME C_4 photosynthesis (Prendergast and Hattersley 1987).

Bicellular microhairs of Baja grass are dumbbell or flask shaped, with a portion of the basal cell sunken below the epidermis into mesophyll or colorless cells (Fig. 6).

DISCUSSION

Analyses of molecular data do not support the hypothesis that Baja grass is a hybrid between *D. littoralis* and *D. spicata* (McDade 1992; Rieseberg et al. 1996). In both ITS and combined chloroplast (*ndhF* + *trnL–trnF*) analyses, Baja grass does not group with any other species but is supported as sister to or a member of *Distichlis* (Figs. 1 and 2). However, three South American endemics, *D. humilis*, *D. laxiflora*, and *D. scoparia*, are resolved

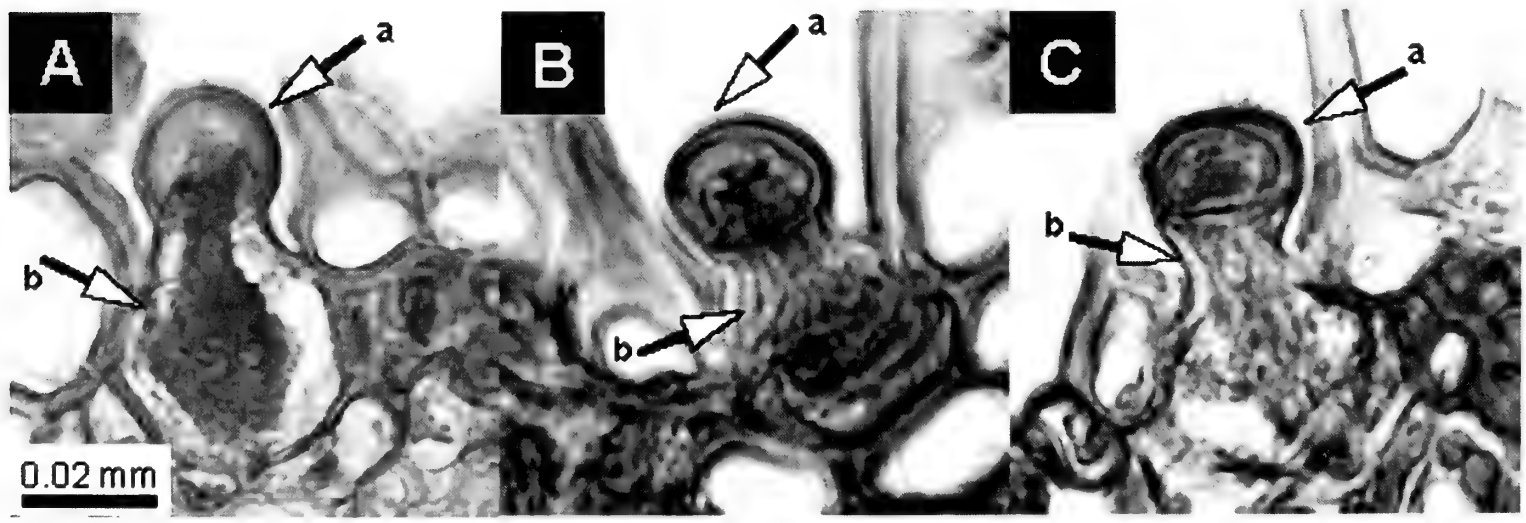

FIG. 6. Bicellular microhairs, A. Baja grass (*Bell 458*), B. *Distichlis spicata* (*Bell 231*), C. *Distichlis littoralis* (*Bell 260*). a = distal cell, b = basal cell. Scale bar applies to A, B, and C.

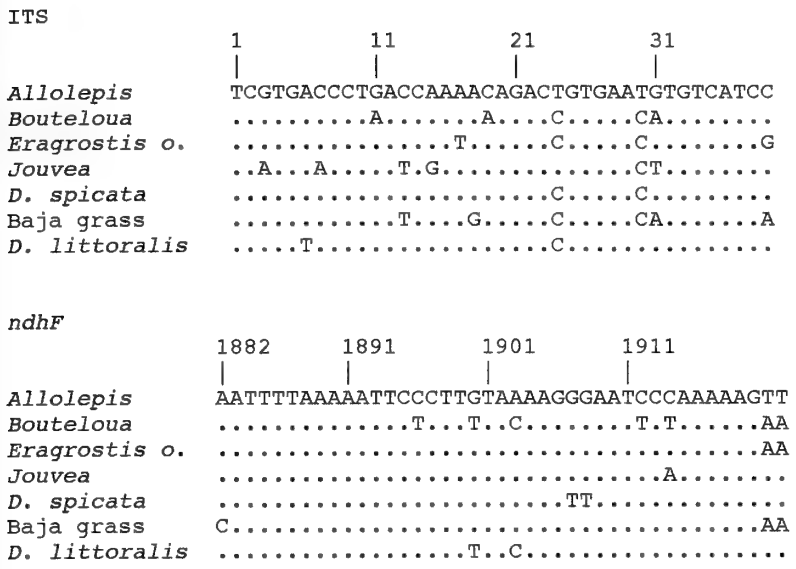

FIG. 7. Patterns of variation in two sections from this study's molecular data sets. Top, the beginning of nuclear ITS; bottom, a relatively variable region of *ndhF*. The first four taxa are the outgroup for this study; they are followed by *D. spicata* (*Bell 231*), Baja grass (*Stephenson 68-304a*), and *D. littoralis* (*Bell 260*).

in conflicting positions by nuclear and chloroplast markers demonstrating that there is adequate signal in these datasets to detect potential reticulation.

A visual comparison of sequence segments from ITS and *ndhF* (Fig. 7) does not reveal the additive pattern that would be predicted if Baja grass were a hybrid between *D. littoralis* and *D. spicata*. If Baja grass were a relatively recent hybrid I would expect to see polymorphisms that were compatible with derivation from *D. littoralis* or *D. spicata*. If the hybridization event occurred in the distant past so that homogenization of ITS alleles had taken place (as is indicated by the finding of nine identical clones), then I would expect that sequences of Baja grass would resemble one or the other of the putative parents. If Baja grass were a hybrid, I would expect that chloroplast sequences would be the same or highly similar to one of the putative parents. As seen in Fig. 7, these are not the patterns that are observed. Variation in sequences from Baja grass does not suggest derivation from either *D. littoralis* or *D. spicata*.

Baja grass has the same blade organization as species of *Distichlis* (Fig. 4). Blades are U-shaped, with vascular bundles separated by furrows and columns of colorless cells. There are few 1st order vascular bundles with narrow xylem elements. There is some variation in the amount of sclerenchyma but its distribution is similar. Dumb bell or flask shaped microhairs are found in Baja grass and all species of *Distichlis* as well as *Eragrostis obtusiflora* and a few other more distantly related halophytic chloridoids. There is evidence that these microhairs are the site of salt secretion in halophytic chloridoids (Oross and Thomson 1982; Amarasinghe and Watson 1988; Warren and Brockelman 1989; Ramadan 2001; Bell and O'Leary 2003). Salt crystals have been observed on the surface of Baja grass blades.

Habitat preferences and anatomical and morphological similarities of the Baja grass to other *Distichlis* species support its inclusion as a new species within the genus. All *Distichlis* species occur in saline or alkaline habitats, are dioecious, have multi-nerved lemmas, Kranz anatomy that predicts NAD-ME type C_4 photosynthesis, numerous papillae on blade surfaces, bulbous bicellular microhairs, columns of colorless cells between vascular bundles, narrow metaxylem elements in 1st order vascular bundles, and relatively few 1st order vascular bundles per blade. Based upon these shared characters and the molecular evidence, this grass is described as a new species of *Distichlis*.

TAXONOMY

Distichlis bajaensis H. L. Bell. sp. nov. (Fig. 8). — Type: MEXICO, Baja California, Municipio de Ensenada, salt marsh in arroyo 1 km SW of Rosarito, area dominated by juncus and salt grasses, heavily grazed by burros and goats, October 1968, *Stephenson 68-304a* (holotype: MSC 216526! [not MSC 216528 or 289874]). Paratype: MEXICO, Baja California, Municipio de Ensenada, southwest of El Nuevo Rosarito, 28°36′40″N, 114°03′03″W, 100 m elevation, broad, dry arroyo with alkaline seeps, growing with *Distichlis spicata* (L.) Greene, *Juncus acutus* L., *Allenrolfea* sp., *Lycium* sp., and *Salicornia* sp., 2 April 2008, *Bell 458*, (BCMEX, MEXU, MO, RSA, UC, US).

Gramen perenne decumbens rhizomatosum stoloniferum ramis intravaginalibus secus stolones, 8–12 cm altum, ligulae pilis linea minuta dispositis, laminis 8–15 mm longis ad collo patentibus, in apicem pungentem sensim decrescentibus, ad faciem adaxialem parum flexis, pilis antrorsis secus margines et faciem abaxialem fascis vascularis medii ad et supra flexuram.

Sprawling, decumbent perennial with rhizomes and stolons, 8–12 cm tall, intravaginal branching along stolons, culms 1 mm in diameter, glabrous, sheaths open, glabrous, with tiny hairs along margins, ligules a minute line of hairs, blades 8–15 mm long, spreading at collar, narrowing gradually to pungent tip, with slight bend toward adaxial side, antrorse hairs along margins and along the abaxial side of the median vascular bundle at and above bend, male inflorescences a small panicle of racemes, inflorescences exserted above blade tips on peduncles of up to 1 cm, flattened pedicels 3–5 mm with toothed margins, 2–5 spikelets per inflorescence, 2–4 florets per spikelet, 1st glume 3 mm, 2nd glume 5 mm, both hyaline with a single nerve, lemmas 7–9 mm with

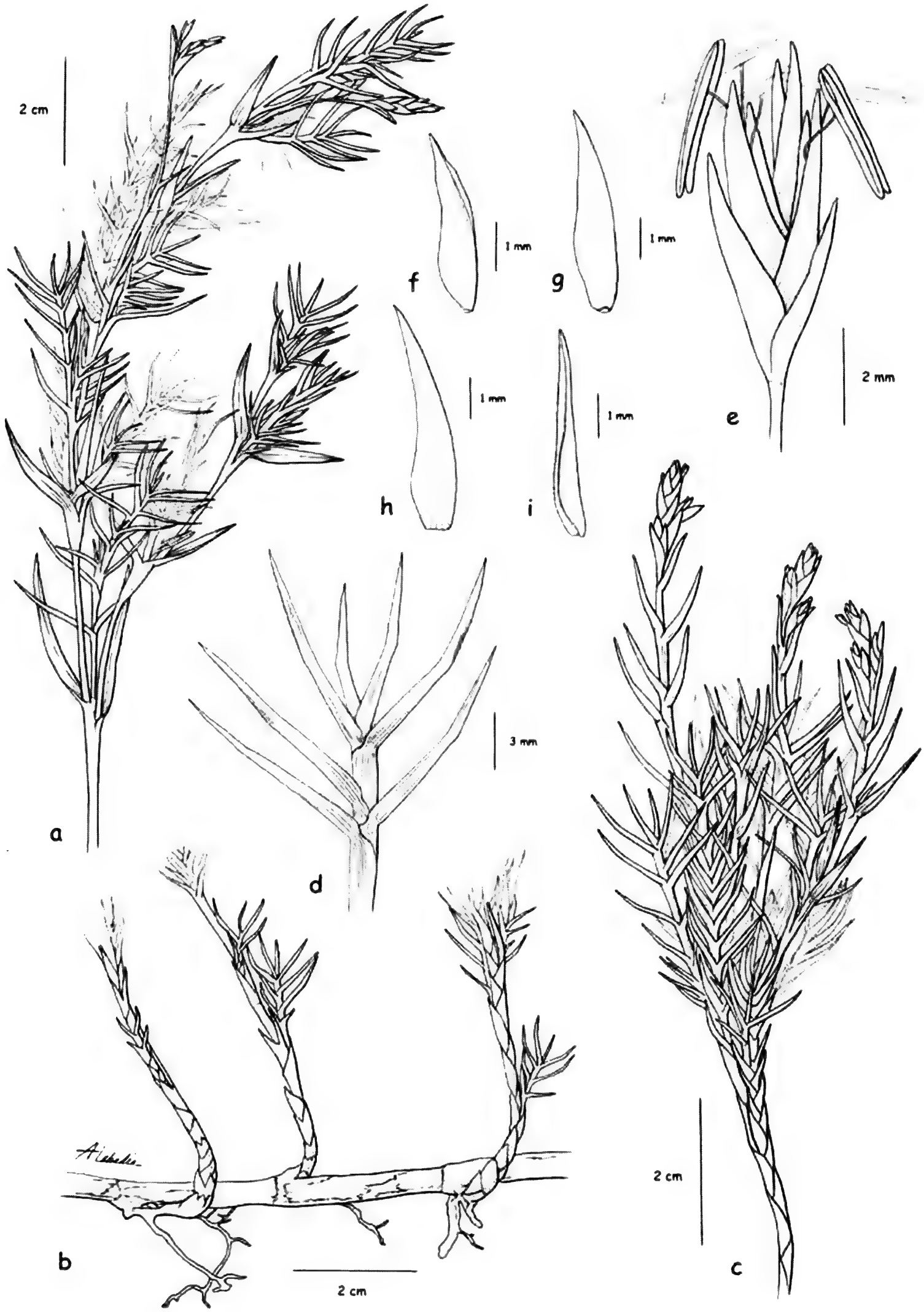

FIG. 8. *Distichlis bajaensis*. a. Female plant habit; b. Rhizome; c. Male plant habit; d. Detail of blades; e. Male spikelet; f. First glume from male spikelet; g. Second glume from male spikelet; h. Lemma from male spikelet; i. Palea from male spikelet. a and c from *Stephenson 68-304a*; b, d – i from *Bell 458*. Illustration by Amanda Labadie.

TABLE 2. COMPARISON OF CHARACTERS THAT CAN BE USED TO DISTINGUISH *DISTICHLIS BAJAENSIS*, *D. LITTORALIS*, AND *D. SPICATA*.

Character	*D. bajaensis*	*D. littoralis*	*D. spicata*
Blade length (cm)	0.8–1.5	<0.8	>2.0
Blade tips	narrow gradually to pungent tip	narrow abruptly to blunt tip	narrow gradually to blunt tip (rarely pungent)
Blade angle from culm	divaricate	divaricate	appressed or divaricate
Blade curve or bend	slight bend toward adaxial side	straight or slight curve toward abaxial side	generally straight
Glumes	present	absent	present
Male inflorescence exserted	yes	no	yes
Plant color	yellowish green	bluish green	bluish green

7–11 indistinct nerves, hyaline, palea slightly shorter than lemma, enclosed within lemma, anthers 2.5–3.5 mm, straw colored (some with purple tinge).

No fresh female inflorescences or caryopses were examined. Stephenson (1971) observed extensive grazing in the collection area and noted "only fragmentary grass specimens could be obtained". He was not able to collect caryopses but provided observations of ovaries and stigmas. Table 2 gives characters that can be used to distinguish between *D. bajaensis*, *D. littoralis*, and *D. spicata*. A distinctive field character is a small bend near the middle of the leaf blade (Fig. 8d). Generally, at and above the bend, short, antrorse hairs occur along the median vascular bundle on the abaxial surface.

Future studies of *D. bajaensis* will focus on the total distribution of this species and the relationship of this species to the rest of *Distichlis*. At present, the species is known from a single large population that appeared to be all or predominately male. It is crucial to learn if other populations exist, the proportions of sexes in those populations, and their proximity to the Arroyo Rosarito population. Although Stephenson found male and female plants during his 1968 collection, John and Charlotte Reeder observed only male plants in 1979 (R. Felger, University of Arizona, personal communication). *Distichlis* species are capable of extensive vegetative reproduction via rhizomes and stolons and highly skewed sex ratios have been observed in many populations (e.g., *D. distichophylla*, Connor and Jacobs 1991; *D. spicata*, Freeman et al. 1976; Eppley et al. 1998). Even though vegetative reproduction occurs, the conservation status of *D. bajaensis* may well be extremely fragile with few or no female plants in existence.

The ITS phylogeny places *D. bajaensis* as sister to the remaining *Distichlis* (Fig. 1). If this is corroborated by future work, *D. bajaensis* will hold a phylogenetic position that is critical to investigating character development and evolution in *Distichlis* by enabling researchers to better understand pleisomorphies vs. apomorphies in the genus.

ACKNOWLEDGMENTS

I thank Carol Annable and Gwyneth Pett for assistance with collecting. Alan Prather, curator of MSC, kindly facilitated a loan of *Stephenson 68-304a* and granted permission for destructive sampling for DNA analysis. Thanks to Amanda Labadie for the illustration, to Mark Garland for the Latin diagnosis, and to Cristina Martinez-Habibe and Gilberto Ocampo for the translation of the Resumen. J. Travis Columbus photographed the blade transection and microhair (Figs. 5A and 6A). Kelly Allred, Lynn Clark, Lucinda McDade, Linda Prince, and Erin Tripp read earlier drafts and provided many helpful suggestions that improved the manuscript. Lon E. Bell gave support and encouragement at every stage of this project.

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APPENDIX 1

LIST OF TAXA SAMPLED

Taxa used as sources of DNA for the molecular phylogeny. See Bell and Columbus (2008) for additional details about *Distichlis* morphology and anatomy. Biogeographical abbreviations used in Figs. 1 and 2 are underlined. GenBank accession numbers (starting with EF or GU) appear in this order: ITS, *trnL–trnF*, *ndhF*. For a few specimens, the *trnL-trnF* sequence was not available and this is designated as 'NA'.

Allolepis texana (Vasey) Soderstr. & H. F. Decker. USA. TEXAS: *Bell 240* (RSA), EF153021, EF156670, EF561646. ***Bouteloua dactyloides*** (Nutt.) Columbus. MEXICO. QUERÉTARO: *Columbus 2329* (RSA), EF153026, EF156675, EF561647. ***Eragrostis obtusiflora*** (E. Fourn.) Scribn. MEXICO. MICHOACÁN: *Bell 314* (RSA), EF196874, EF196902, EF561648. ***Jouvea pilosa*** (J. Presl) Scribn. MEXICO. JALISCO: *Bell 247* (RSA), EF153057, EF156706, EF561649. ***Distichlis acerosa*** (Speg.) H.L. Bell & Columbus (=*Monanthochloë acerosa* (Griseb.) Speg.). ARGENTINA. LA RIOJA: *Bell 389* (RSA), LR ARG, EF196897, EF196924, EF561671. CATAMARCA: *Bell 392* (RSA), CA ARG, EF196898, EF196925, EF561672. ***Distichlis australis*** (Speg.) Villamil. ARGENTINA. RIO NEGRO: *Bell 330* (RSA), RN ARG, EF196875, EF196903, EF561650. SANTA CRUZ: *Bell 357* (RSA), SC ARG, EF196876, EF196904, EF561651. ***Distichlis bajaensis*** H.L. Bell. MEXICO. BAJA CALIFORNIA: *Bell 458* (RSA), GU562862, NA, GU562863; *Stephenson 68-304a* (MSC), ITS Clones 1, 2, 3, 5, 6, 7, 8, 9, 10 GU562864, ITS Clone 4 GU562865, GU562867, GU562866. ***Distichlis distichophylla*** (Labill.) Fassett. AUSTRALIA. VICTORIA: *Cochrane 1198* (MEL), VIC AUS, EF196877, EF196905, EF561652. SOUTH AUSTRALIA: 12 October 2003, *Walsh s. n.* (12 October 2003), SA AUS, EF196878, EF196906, EF561653. ***Distichlis eludens*** (Soderstr. & H.F. Decker) H.L. Bell & Columbus, (=*Reederochloa eludens* Soderstr. & H.F. Decker). MEXICO. SAN LUIS POTOSÍ: *Bell 250* (RSA), SLP MEX, EF153077, EF156726; *Columbus 4133* (RSA), SLPc MEX, EF196901, EF196928, EF561676. ***Distichlis humilis*** Phil. ARGENTINA. JUJUY: *Bell 405* (RSA), JU ARG, EF196879, EF196907, EF561654. BOLIVIA. DEPARTAMENTO ORURO: *Peterson 12833* (US), BOL, EF196880, NA, EF196908. ***Distichlis laxiflora*** Hack. ARGENTINA. BUENOS AIRES: *Bell 367* (RSA), BA ARG, EF196881, EF196909, EF561656. CÓRDOBA: *Bell 381* (RSA), CO ARG, EF196882, EF196910, EF561657. ***Distichlis littoralis*** (Englem.) H.L. Bell & Columbus (=*Monanthochloë littoralis* Engelm.). USA. TEXAS: *Bell 236* (RSA), coTX USA, EF153065, EF156714, EF561673. CALIFORNIA: *Bell 260* (RSA), coCA USA, EF196900, EF196927, EF561674. ***Distichlis palmeri*** (Vasey) Fassett ex I. M. Johnst. MEXICO. SONORA: *Columbus 3586* (RSA), SOc MEX, EF196883, EF196911, EF561658; *Felger 91-39* (RSA), SOf MEX, EF196884, EF196912, EF561659. ***Distichlis scoparia*** (Nees ex Kunth) Arechav. ARGENTINA. RIO NEGRO: *Bell 328* (RSA), RN ARG, EF196885, EF196913, EF561660. CHILE. VALPARAISO: *Bell 374* (RSA), VA CHI, EF196886, EF196914. EF561661. ***Distichlis spicata*** (L.) Greene. USA. CALIFORNIA: *Bell 231* (RSA), inCA USA,

EF153040, EF156689, EF561662; *Bell 259* (RSA), coCa USA, EF196890, EF196918, EF561665. TEXAS: *Bell 237* (RSA), TX USA, EF196887, EF196915, EF561663. VIRGINIA: *Bell 290*, VR USA, (RSA), EF196892. EF196920, EF561667. CANADA. BRITISH COLUMBIA: *Bell 277* (RSA), BC CAN, EF196891, EF196919, EF561666. MEXICO. COAHUILA: *Bell 245* (RSA), CO MEX, EF196888, EF196916, EF561664. ARGENTINA. CHUBUT: *Bell 340* (RSA), CH ARG, EF196893, EF196921, EF561668. CHILE. VALPARAISO: *Bell 375* (RSA), VA CHI, EF196895, EF196922, EF561669. PERU. REGION LAMBAYEQUE: *Columbus 3432*, (RSA), PERU, EF196896, EF196923, EF561670.

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CHENOPODIUM LITTOREUM (CHENOPODIACEAE), A NEW GOOSEFOOT FROM DUNES OF SOUTH-CENTRAL COASTAL CALIFORNIA

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ABSTRACT

Chenopodium littoreum is described as new. It had been incorrectly cited in the past as *C. carnosulum* Moq. var. *patagonicum* (Phil.) Wahl, a variety of the South American *C. carnosulum*. However, **C. littoreum** differs from the *C. carnosulum* complex in having narrowly elliptic to lanceolate and mostly unlobed leaves, consistently five stamens per flower, and seeds that are invariably horizontal. **Chenopodium littoreum** is similar to another South American taxon, *C. patagonicum* Phil. (=*C. philippianum* Aellen), but the latter differs in having basally lobed leaves, sepals fused above the middle, and generally one or two (rarely five) stamens. **Chenopodium littoreum** has a range currently known only from coastal dunes of San Luis Obispo Co. and Santa Barbara Co. of the Central Coast of California, plus a single historic collection from Los Angeles Co. of the South Coast of California.

Key Words: *Chenopodium*, *C. carnosulum* var. *patagonicum*, *C. patagonicum*, *C. philippianum*, Chenopodiaceae, dune flora, coastal goosefoot.

Chenopodium (Chenopodiaceae; Amaranthaceae *sensu* APG III 2009) is a large genus of approximately 100 species of mostly temperate plants, with a worldwide distribution. It is segregated from the related genus *Dysphania* (ca. 32 species) in recent treatments (Clemants and Mosyakin 2003a, b). Although many species of *Chenopodium* are weeds, some are economically important, such as the pseudo-grain *C. quinoa* of South America (Mabberley 2008).

The preparation of the *Chenopodium* treatment (Clemants and Benet-Pierce in preparation) for the second edition of *The Jepson Manual* necessitated the resolution of issues left pending by the untimely death of Dr. Steve Clemants of the Brooklyn Botanic Garden. One major issue was the taxon *Chenopodium carnosulum* Moq. var. *patagonicum* (Phil.) Wahl, several specimens of which had been cited as occurring (and presumably naturalized) in San Luis Obispo and Santa Barbara counties, California (Wilken 1993). After reviewing the literature and observing numerous specimens and specimen images, we are convinced that the California taxon in question does not correspond to *Chenopodium carnosulum* Moq., nor to *C. patagonicum* Phil. (*C. philippianum* Aellen; see below), and therefore has been an ongoing case of misidentification.

We propose here that what was previously identified as *Chenopodium carnosulum* var. *patagonicum* is actually an undescribed, new species. We presume it to be native and endemic to California, as specimens of this taxon have not been found elsewhere.

Chenopodium littoreum Benet-Pierce & M. G. Simpson, sp. nov. (Fig. 1).—Type: USA, California, San Luis Obispo Co., road along Jack Lake, ca. 9 km south of Arroyo Grande, ca. 16 m, 35.03858°N, 120.60378°W, 15 May 1966, *R. F. Hoover 9856* (holotype: OBI 17235; isotypes: CAS 473439, 473440, 473441).

Paratypes (see Fig. 1F, G for locality map): USA. CALIFORNIA. **Los Angeles Co.**: Playa del Rey, 33.96184°N, 118.4468°W, 14 May 1904, *G. C. Grant s.n.* (DS 91772). **San Luis Obispo Co.**: Oceano, 35.0946°N, 120.622327°W, 30 April 1910, *G. F. Condit s.n.* (UC 455220); Oceano Dunes, 35.09456°N, 120.622327°W, 30 May 1931, *R. Hoffman 420* (CAS 189558); Oso Flaco Lake, 35.02941°N, 120.62756°W, 13 May 1950, *L. S. Rose 50116* (CAS 367246, RSA 63058, UC 942915); Morro Bay, 35.37257°N, 120.863926°W, 9 June 1967, *R. F. Hoover 10629* (OBI 17236); Morro Bay, 35.37257°N, 120.863926°W, 29 June 1969, *J. R. Potter 51* (OBI 4176); Little Coreopsis Hill, 35.03433°N, 120.615°W, 25 May 1980, *A. P. Griffiths s.n.* (OBI 56356); Black Lake, Highway 1, 35.05885°N, 120.609709°W, 25 April 1985, *D. Keil 18563* (OBI); Los Osos, 35.31548°N, 120.86648°W, 9 June 1985, *D. Keil 18790* (OBI). **Santa Barbara Co.**: SBC Vandenberg Air Force Base, 34.79311°N, 120.621247°W, 23 August 1996, *D. Keil 25849* (OBI 67573); North Base, 34.74747°N, 120.62801°W, 23 August 1996, *D. Keil 25947* (OBI 67553).

Chenopodium littoreum differt a *C. carnosulum* Moq. foliis integerrimis anguste ellipticis lanceolatis vel late lanceolatis plerumque non-lobis basi cuneatis, apice mucronulatis, 5 stamenibus, et semenibus complanatis; differt a *C. patagonicum* Phil. et *C. philippianum* Aellen foliis integerrimis anguste ellipticis lanceolatis vel late lanceolatis plerumque non-lobis, calycis ulterioribus separatis, et 5 stamenibus.

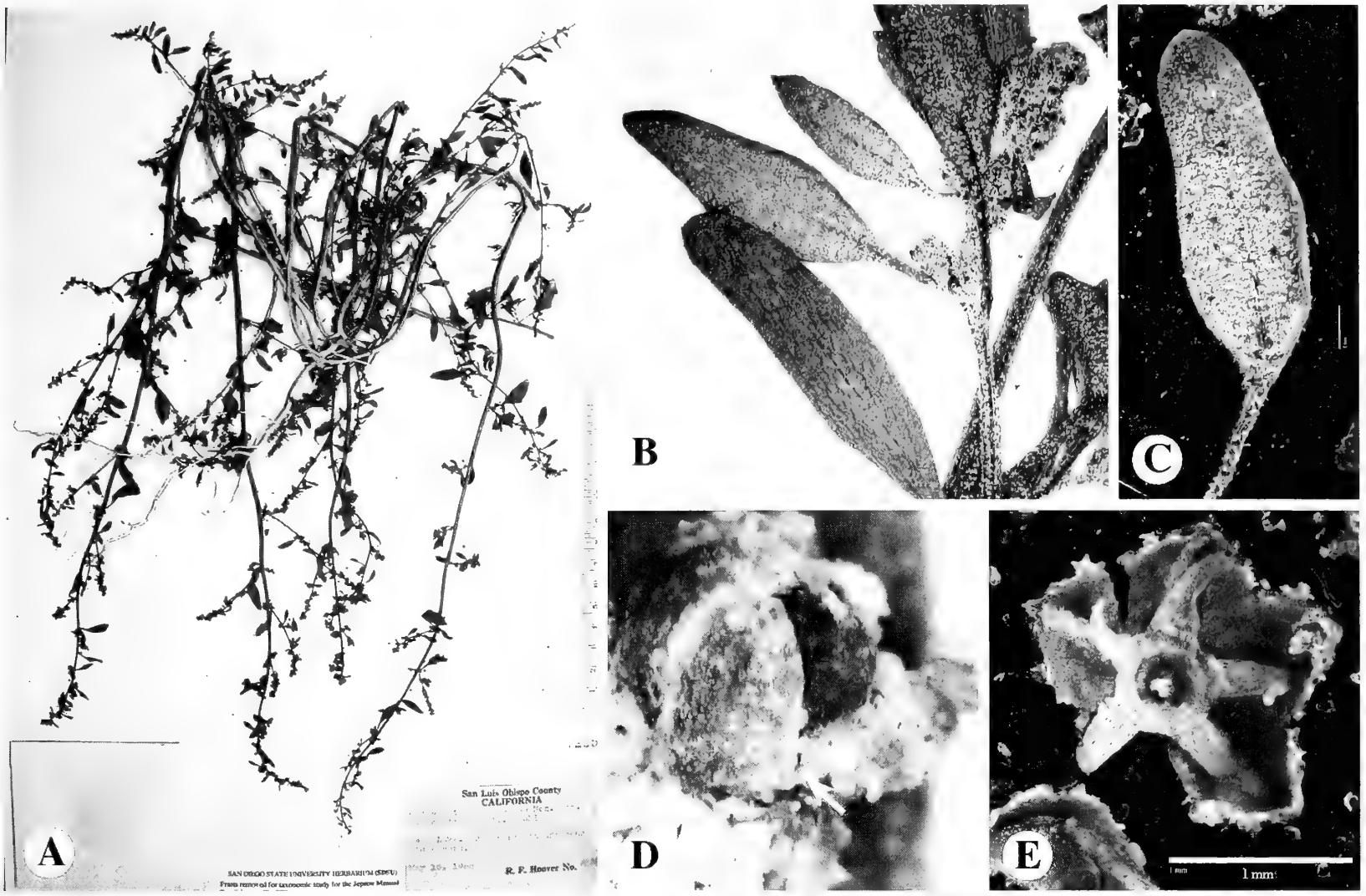

FIG. 1. *Chenopodium littoreum*. A. Herbarium specimen (OBI 17235, holotype). B. Specimen (OBI 67553, paratype). Close-up of leaves, showing narrowly elliptic to lanceolate shape. C. Specimen (CAS 473441, isotype). Single leaf close-up; note farinose surface. Scale bar = 1 mm. D, E. Specimen (OBI 17235, holotype). D. Fruit, showing calyx lobes distinct almost to base (arrow). E. Flower, removed, showing five stamen filaments. Scale bar = 1 mm. F. Distribution map of known collections. G. Close-up of specimen localities in Santa Barbara and San Luis Obispo counties.

Chenopodium littoreum differs from *C. carnosulum* Moq. by having entire, narrowly elliptic, lanceolate, or widely lanceolate, mostly non-lobed, basally cuneate leaves, apex mucronulate, 5 stamens, and horizontal seeds; it differs from *C. patagonicum* Phil. and *C. philippianum* Aellen in having entire, narrowly elliptic, lanceolate, or widely lanceolate, mostly non-lobed leaves, with calyx lobes distinct to near base, and 5 stamens.

Annual prostrate herb, branched from base, forming mats to ca 4 dm in diameter. **Leaves** alternate; petioles 5–9 mm long; blades narrowly elliptic, lanceolate, or broadly lanceolate, rarely basally lobed, 6–15 (20) mm long, 3–8 mm wide,

light green; base cuneate, apex acute, obtuse, or rounded, often mucronulate, farinose adaxially, densely farinose abaxially. Inflorescence of glomerules up to 7 mm wide, in axillary and terminal spikes and panicles, 1–15 cm long; bracts leaflike. **Flowers** perfect, radial, approximately 1 mm in diameter; perianth uniseriate; calyx synsepalous, with five lobes, distinct to near base, lobes apically obtuse, densely farinose abaxially. **Stamens** five, distinct, whorled, antisepalous; filaments terete, yellow, with laterally dehiscent, dithecal, subbasifixed anthers. **Gynoecium** syncarpous, hypogynous; ovary superior, with two stigmas. Placentation basal with one curved ovule. **Fruit** an achene, horizontal, dark brown, lenticular, margin rounded, approximately 1 mm in diameter; fruit wall minutely tuberculate to smooth, attached to the seed, but becoming loose at maturity. **Seeds** 0.9–1 mm in diameter, perispermous; seed coat smooth, black-brown to red.

Distribution and habitat: *Chenopodium littoreum* is currently known from dunes of a narrow coastal strip of the Central Coast of California (San Luis Obispo and Santa Barbara counties), and one collection from the South Coast of California (Los Angeles Co.; Fig. 1F, G).

Phenology: *Chenopodium littoreum* appears to flower and fruit from late April to as late as August.

Etymology: The specific epithet, *littoreum*, Latin (pronounced li-TOR-e-um), translates as "of the seashore," in reference to the coastal distribution of this species.

Suggested common name: Coastal Goosefoot.

Discussion

California collections of *Chenopodium littoreum*, described here, have mostly been identified as *Chenopodium carnosulum* Moq. var. *patagonicum* (Phil.) Wahl (basionym *C. patagonicum* Phil.), purportedly a Californian variety of an otherwise mostly South American species. However, the species *C. carnosulum* is markedly different in a number of features from *C. littoreum*.

Christian Horace Bénédict Alfred Moquin-Tandon described *Chenopodium carnosulum* in 1849. It is mostly found in the southernmost tip of South America, in Chile and Patagonia in Argentina, but specimens have been cited from Peru and Mexico. Examination of an on-line image of the holotype of *C. carnosulum* Moq. (K 583167, Port Gregory, Patagonia, Argentina; Fig. 2A) shows a plant with leaves that are relatively small, rhombic-deltoid, and strongly lobed; this is in contrast to the elliptic or lanceolate, mostly unlobed leaves of *C. littoreum* (Fig. 1A–C). Physical examination of other specimens of *C. carnosulum* (UC 559383; GH 257655, 257651, 257652; and GH (Mexia 7960, not accessioned; Fig. 2 B–E) and of the infraspecies *C. carnosulum* Moq. var. *scabricaule* (Speg.) Aellen & Just (GH 257656) all show similar features. The leaves of all of these specimens are small, rhombic-deltoid and strongly lobed (elliptic to lanceolate or widely lanceolate and mostly unlobed in *C. littoreum*); the flower has only one stamen or occasionally 2 (consistently 5 in *C. littoreum*); many of the seeds are vertical or oblique (consistently horizontal in *C. littoreum*); and the fruit wall is often mottled (mottling absent in *C. littoreum*). In addition, the description of *Chenopodium carnosulum* Moq. from the protologue (Moquin-Tandon 1849) states: "Folia 3–4 lin. [=6.3–8.4 mm] longa (incl. petiolo 1/2–1 lin. [=1–2.1 mm]), 1 1/2–2 lin. [=3.2–4.2 mm] lata, subcarnosa; superiora rhombeo-deltoidea ...." This description of the leaves as rhombic-deltoid with a length:width ratio of approximately two substantiates our observations of images and specimens of this taxon. In summary, the significant disparities between *C. carnosulum* Moq. and the taxon described here definitively rules out any possible identity between the two.

Given that the basionym for Wahl's taxon is *C. patagonicum* Phil., we investigated the features of that taxon in comparison to *C. littoreum*. The original description by Philippi (1895) of *C. patagonicum* reads: "foliis ... integerrimis, ovatis seu oblongo-triangularibus, basi sub truncates vel trapezoideis, interdum basi utrinque unidentatis...," translated as "the leaf is entire, ovate or oblong-triangular with base subtruncate, or [leaf] trapezoidal, sometimes basally one-toothed from both sides." These characters are different from the narrowly elliptic to widely lanceolate (base cuneate) leaves of *C. littoreum*, which cannot be described as trapezoidal or subtruncate. The accompanying description in Spanish by Philippi just below the Latin one, says "su lamina 21 milímetros de lonjitud i [sic] 15 milímetros de anchura, pero la mayor parte de las hojas tienen la mitad de ese tamaño ..." ("its blade 21 mm long by 15 mm wide but the majority of the leaves are closer to half of this size"). The measurements of 21mm by 15 mm are inconsistent with the leaf length of *C. carnosulum* (ca. 6 to 8 mm) and are not those of an elliptic to widely lanceolate leaf either, as in the Californian *C. littoreum*. In the original description, the leaves of *C. patagonicum* (Philippi 1895) resemble those of *C. carnosulum* in shape, but are apparently larger in size.

Additional evidence of the distinctiveness of *C. littoreum* comes from synonomy. Aellen (1929) and Aellen and Just (1943) combined three previously described Argentinian taxa - *C. fuegianum* Speg. (1896), *C. patagonicum* Phil. (1895), and *C. scabricaule* Speg. (1902) (the last having three varieties) with *C. carnosulum* Moq. (1849), which has nomenclatural priority. Thus, these authors considered *C. patagonicum* Phil. to

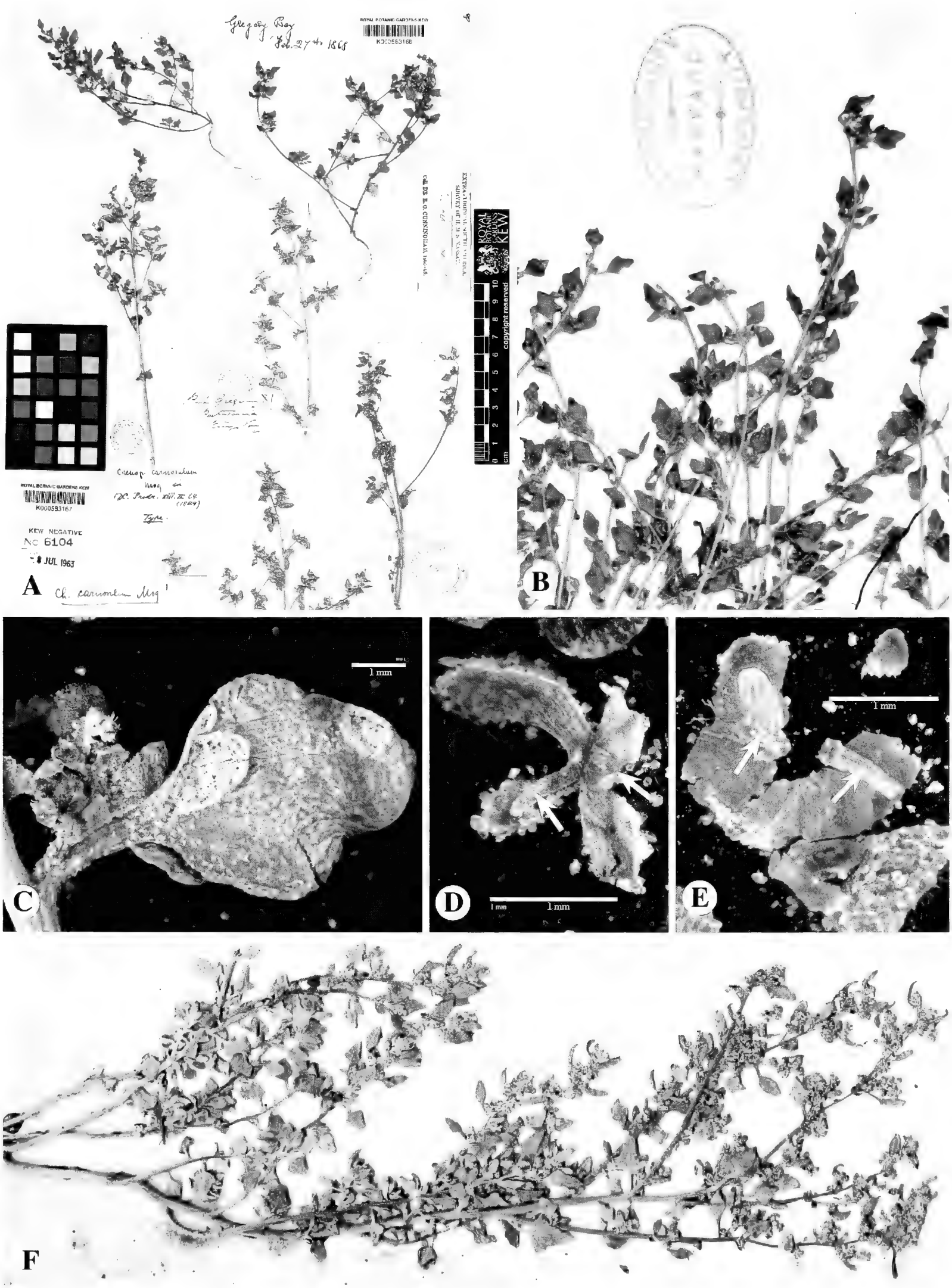

FIG. 2. *Chenopodium carnosulum* Moq. A. Holotype (K 583167). Note relatively short, rhomboid to deltate, basally lobed leaves. B–E. Specimen, Mexia 7960 (GH, *s.n.*). B. Close-up of shoot, showing similar, rhomboid leaves. C. Single leaf, showing rhomboid shape with two lateral lobes. Scale bar = 1mm. D, E. Close-up of flower remains, showing calyx lobes distinct nearly to base and only two stamens (arrows). Scale bars = 1 mm. F. *C. carnosulum* (*Chenopodium parryi* Standl.) specimen C. Parry 780 (central Mexico, 1878, MO 46467, isotype). Note identical, rhomboid-trapezoid leaves.

be the same taxon as *C. carnosulum*, which, as we already have shown, is quite distinct from *C. littoreum*. These authors presumably thought that any variation between these three species, in a genus well known for its lack of definite and stable leaf characters, was insufficient to warrant separate species status from *C. carnosulum*.

Aellen, having revised the genus in the American continent, pointed out that the original collection of *C. carnosulum* did not come from California, as Moquin-Tandon had noted, but from Port Gregory, Patagonia. Aellen (1929) annotated the type specimen collected by O. Cunningham, the same that Moquin-Tandon had identified as the holotype of *C. carnosulum* Moq. (K 583167). Aellen was clear in his opinion of this: "Moquin made a mistake when he stated, in the 'Prodomus', California as the native country of the original plant. The exemplary originates from Patagonia (Port Gregory). This lead to the fate of the species being sealed in the South American literature. North American botanists were certainly mystified by *Ch. carnosulum* Moq., as it couldn't be found in California. S. Watson (l.c.) treated it as a 'doubtful species.' Standley (l.c.) mentioned it from Mount Orizaba, Mexico; yet the identification is not certain." (Aellen 1929, translation by D. Pierce-Knies, personal communication).

In order to ascertain the presence of the South American *C. carnosulum* in North America, we studied other species that have been associated with *C. carnosulum*. One of them, *C. parryi* Standl., was for a time an accepted taxon. The type specimen from Mexico (MO 46467, C. Parry 780, central Mexico, 1878; Fig. 2F) shows a species with a trilobed leaf much like *C. carnosulum*, described by Standley as "... leaf-blades triangular or triangular-rhombic in outline, 3–5 mm. long, 3–4 mm. broad, 3-lobed, ..." (Standley 1916). Wahl (1965) also considered this species, stating "The type (no other collection has been referred to it) fits in geographically with the other two Mexican records even if these were difficult to place with any ... *C. Parryi* Standley seems to be the same as *C. carnosulum* Moq. var. *carnosulum*" (Wahl 1965). And we concur, as the type from MO (Fig. 2F) shows the same rhomboid, basally lobed leaf as in *C. carnosulum*, evidently different from that of *C. littoreum*. Thus we confirmed the presence of *C. carnosulum* in North America, but not in the United States.

H. A. Wahl, who revised the genus *Chenopodium* in North America (Wahl 1954, 1965) had recognized the California taxon as puzzling, citing several specimens from CAS that "when I examined them in 1955, could not be placed with any known North American species. These were from sand dunes or similar habitats along or near the coast in San Luis Obispo and Santa Barbara counties, California" (Wahl 1965, p. 137). Wahl (1965) believed that the California specimens in question were *C. patagonicum*, which he then reduced in rank to *C. carnosulum* var. *patagonicum*. Wahl based his opinion solely on what he described as a photograph of the type of *C. patagonicum*, which he said "is such an exact match for the California plants as to leave no doubt as to their inclusion with this species" (Wahl, 1965, p. 138). As representatives of this taxon, Wahl cites one Chilean specimen (*Bauchtien s.n.*, in part, Feb. 1903, US; this specimen not listed on the US database); two Mexican specimens (*Seaton 184*, 6 Aug. 1891, GH; this specimen not listed on the Harvard University Herbaria database; *Balis B5503*, 22 Sept. 1938, UC), and several California specimens (*Eastwood 789*, 2 July 1906, CAS; *Hoffmann s.n.*, 29 March 1939, CAS; *Condit s.n.*, 30 April 1910, UC; *Hoffmann 420*, 30 May 1931, CAS; and *L. S. Rose 50116*, 13 May 1950, CAS, UC). However, his conclusions are puzzling, given the disparity in leaf morphology (let alone stamen number) between *C. littoreum* and *C. carnosulum*. We have not seen the specific Chilean specimens he mentioned, but we have examined other specimens of *C. carnosulum*. Having seen all of the same specimens of California collections, we firmly believe they do not correspond to *C. carnosulum*. Wahl, however, treated the California taxon as a variety of *C. carnosulum*, presumably on account of the differences he observed and because *C. patagonicum* had already been treated as a synonym of the former by Aellen (1929) and Aellen and Just (1943).

We have been unable to physically examine specimens of *C. patagonicum* Phil., but we have now seen an image of the type (SGO 38811; Fig. 3). The type specimen does look similar to *C. littoreum* in leaf morphology in that some leaves are narrowly elliptic to widely lanceolate. However, most leaves, in particular the mature ones, are "trullate" in appearance, i.e., rhombic with a more elongate upper half, with two, small lobes near the base, and a mostly rounded apex (Fig. 3B). Thus, leaf morphology of *C. patagonicum* is somewhat different from that of *C. littoreum*, and intermediate to that of a typical *C. carnosulum* (Fig. 2). It is plausible that it was the picture of this plant, identified as *C. patagonicum* Phil., that convinced Wahl that the Californian plants were equivalent, introduced from South America.

From the SGO 38811 image of the *C. patagonicum* type, we noted that this specimen had been annotated as *C. philippianum* (A. Marticorena, annotated 2000; Fig. 3C). In addition, *C. patagonicum* has been treated as a synonym of *C. philippianum* in at least one recent treatment (Marticorena 2008). If indeed these two taxa are equivalent, we do not understand why *C. patagonicum* Phil. (1895) would not have

FIG. 3. *Chenopodium patagonicum* Phil. Type specimen (SGO 38811). A. Whole herbarium sheet, B. Close up of larger plant (at left on sheet). Note leaves varying from narrowly elliptic to widely trullate, with two, small lobes near base. Scale bar = 1 cm. C. Close-up of herbarium labels. Note original designation as *C. patagonicum* Phil., annotated as *Chenopodium philippianum* Aellen by A. Marticorena (2000).

nomenclatural priority over *C. philippianum* Aellen (1929). This discrepancy we hope to address in a later study in conjunction with our Chilean colleagues at SGO.

Because *C. philippianum* looks superficially similar to *C. littoreum*, and indications are it may be equivalent to *C. patagonicum*, it was particularly important to thoroughly investigate the former from specimens. We have physically examined *C. philippianum* (GH 257649; Fig. 4A–C), the same specimen Wahl had also examined and which he had determined to be different from the California collections. We found the leaves to resemble *C. patagonicum*, being generally rhomboid and lobed, although they are much larger and with lobes much less pronounced than *C.*

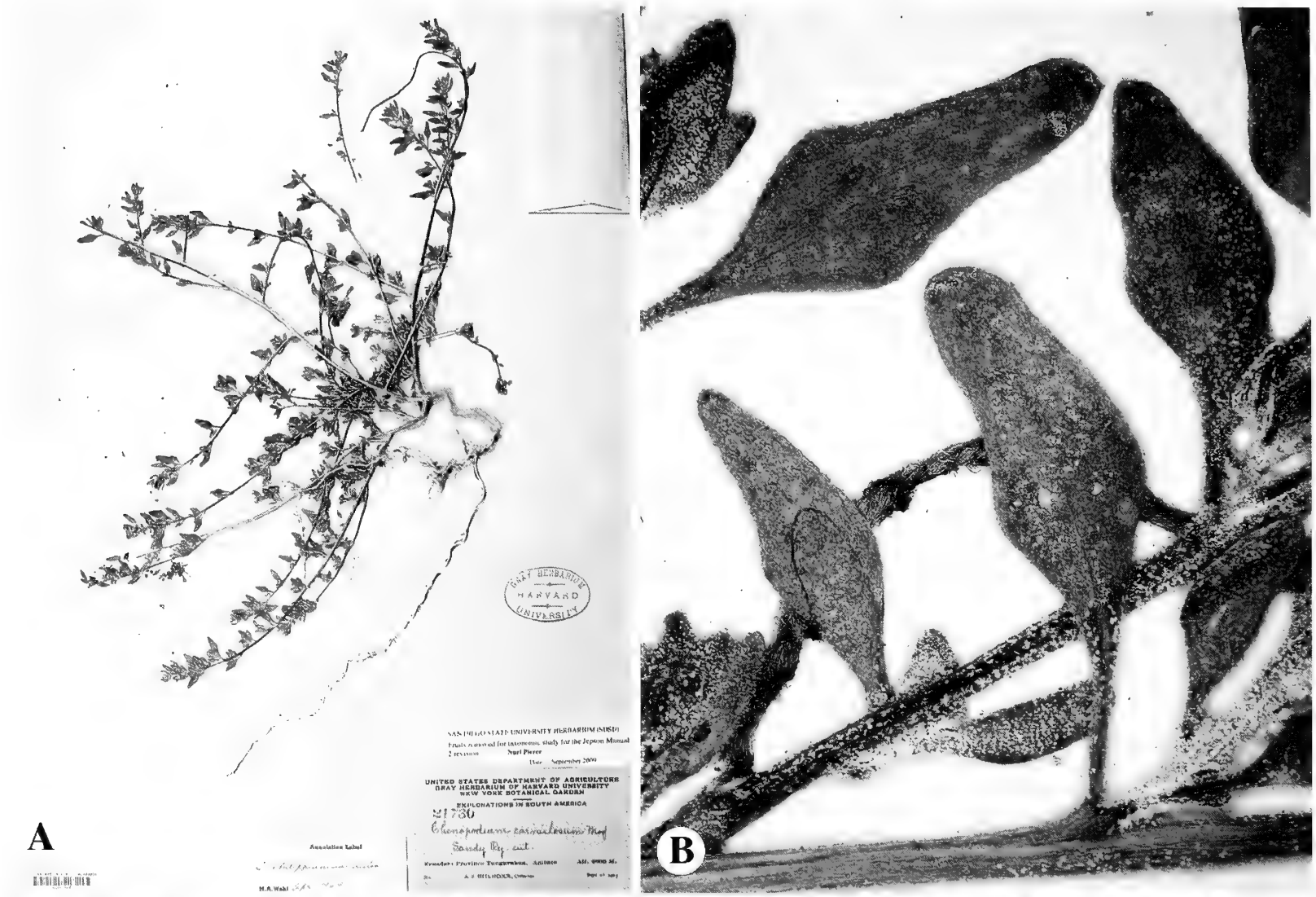

FIG. 4. *Chenopodium philippianum* Aellen. A–C. Specimen GH 257649. A. Herbarium sheet. B, C. Leaves, showing somewhat trullate to widely lanceolate shape, with slight lobbing near base. Scale bar = 1 mm. D, E. Specimen GH 21730. Scale bars = 1 mm. D. Fruit, showing calyx fused (arrow) more than halfway to apex. E. Fruit, showing remains of two stamens (arrows).

carnosulum. We have been able to ascertain that the leaf apices are rounded to obtuse and generally not mucronulate, which is often the case in *C. littoreum*. In the two type specimens of *C. philippianum* (K 583181 and K 58382, both images available on line), the leaves are even more strongly lobed than the specimen we physically examined, but they probably represent more mature plants. *C. philippianum* also has a variable number of stamens (mostly 2–3, occasionally 5) (GH 21730; Fig. 4E). In addition and perhaps more significantly, the sepals of *C. philippianum* are fused to half or more than half of their length (Fig. 4D), whereas in *C. littoreum* the calyx is fused well less than half its length (Fig. 1D), calyx fusion being somewhat useful diagnostically in *Chenopodium*. Thus, we can rule out this species being the same as the Californian taxon on the basis of the leaf shape and apex, calyx fusion, and stamen number (Fig. 4). In general, though, this species does show stronger similarities with *C. littoreum* than do any taxa of the *C. carnosulum* complex, and future molecular work could better elucidate their relationship. To further explore the *C. patagonicum* type, we asked the curator of SGO in Santiago, Chile, to examine the type of *C. patagonicum* (SGO 38811; Fig. 3). Dr. M. Muñoz reported the

specimen having 2 and 5 stamens and a calyx fused to around the middle (personal communication). These findings would support the consideration that *C. patagonicum* Phil. and *C. philippianum* Aellen are the same species. We also reviewed the diagnosis of *C. philippianum* by Aellen (1929). Aellen had problems identifying the material from which he diagnosed this species: "The labeling of the Philippianum material is extremely difficult. To approximate the species is only indirectly possible. The Washington original material of Cordillera de Talca is a very incomplete, small specimen, which can't be accurately identified; the one from Berlin is a little more complete, but does not feature any fully developed seeds ... Philippi, seemingly, never published his *Ch. Andinum* ..." (translation by D. Pierce-Knies, personal communication).

It is plausible that Aellen (1929) described *C. philippianum* as a new species (even given the poor material he had seen), unaware that it was equivalent to *C. patagonicum*. In the past, he had incorrectly accepted *C. patagonicum* to be a synonym of *C. carnosulum* even if he had done this while issuing a warning that the synonomy of *C. carnosulum* could be in doubt: "Assumedly, it [*C. carnosulum*] was newly characterized by Phlilippi or Spegazzini; it still needs to be established with certainty whether it is the same as *Ch. patagonicum* Phil. or *Ch. fuegianum* Speg. or *Ch. Scabricaule* Speg." (Aellen 1929, translation by D. Pierce-Knies, personal communication). We have recently seen an image of *C. fuegianum* (SGO 59002), which is now identified as *C. carnosulum var carnosulum*, *C. carnosulum* having priority over *C. fuegianum*. Aellen's concerns also give further credence that these two species, *C. philippianum* and *C. patagonicum*, could be the same.

On the other hand, when Wahl examined the California collections, specimens that had been sent to Wahl by R.F. Hoover from San Luis Obispo, the notion that *C. littoreum* could be a new species did occur to him. He wrote (Wahl 1965): "The possibility of these representing an undescribed species was considered but the known occurrence on the west coast of varieties of species native in the drier and colder parts of southern and western South America [*C. macrospermum* Hook. f. var. *farinosum* (Wats.) J. T. Howell, *C. chenopodioides* (L.) Aellen var. *Degenianum* (Aellen) Aellen and var. *Lengyelianum* (Aellen) Aellen] suggested a possible similar relationship for these relatively restricted plants." Wahl never confirmed this relationship. We have been able to determine that the above naturalized *Chenopodium* species for the most part have vertical seeds, and probably are not comparable at all to *C. littoreum*; they were presumably cited as an analogy, indicating that because other South American species have become established in California, what we are calling *C. littoreum* could have been as well.

Thus, although it was presumably a picture of the type of *C. patagonicum* that convinced Wahl of its equivalence to what we are describing as *C. littoreum*, we can only rely on the facts: 1) that *C. patagonicum* is described as having "ovate or oblong-triangular with base subtruncate, or [leaf] trapezoidal, sometimes basally one-toothed from both sides, 21 mm long by 15 mm wide" in the protologue (Philippi 1895), agreeing more with the leaf shape of *C. carnosulum* and *C. philippianum* but not with *C. littoreum*; 2) that the type of *C. patagonicum* shows differences in leaf morphology from *C. littoreum* in the former being trullate in shape with basal lobes; 3) that *C. patagonicum* has been considered a synonym of *C. carnosulum* by some authors (Aellen 1929; Aellen and Just 1943), a taxon quite different from *C. littoreum*; and 4) that *C. patagonicum* is apparently equivalent to *C. philippianum*, a taxon that we have been able to show differs from *C. littoreum* in having stamen number 2–3 or occasionally 5, a more extensive sepal fusion, and differences in leaf morphology. Therefore, we do not believe that *C. patagonicum* Phil., nor by extension *C. carnosulum* Moq. var. *patagonicum* (Phil.) Wahl, nor *C. philippianum* Aellen are the same taxon as *C. littoreum*. No other South American taxa that we know have been associated at any point with these species' characteristics. We have thoroughly reviewed every species at one time associated with *C. carnosulum* and C. *patagonicum*. We have reviewed Chilean (Marticorena, 2008; Reiche, 1911) and Argentinean (Toloaba, 2006) keys to *Chenopodium* and have found no other species that would fit the description of *C. littoreum*. In particular, it is the highly restricted range of *C. littoreum*, in the absence of any other likely candidate in *Chenopodium* keys for South America, Baja California (Wiggins, 1980), or neighboring North American states (Clemants and Mosyakin 2003a), plus its differences with the above-mentioned species, that supports the conclusion that it is endemic, particularly in a region well known for dune endemic vegetation (D. Keil California Polytechnic State Univ., personal communication).

In conclusion, the Californian *Chenopodium littoreum* described here does not conform to any of the South American taxa that have been associated with it nor to any other we have separately considered, and its narrow range makes it unlikely that it should be. *Chenopodium littoreum* is also unlike any other North American species in the genus. Although it shares some characters with other *Chenopodium* species found here, with the usual horizontal seed and five perianth parts, none of these taxa is prostrate. Other *Chenopodium* species in North America that are either prostrate or somewhat decumbent

have vertical or vertical and horizontal seeds and have usually one or two stamens, or other differing vegetative or floral characters.

We end with this quote from Wahl (1954): "No group of plants of comparable size and wide distribution known to the writer has suffered the lack of understanding of the taxa involved as has the genus *Chenopodium* ... The reasons for this lie in (1) the ecological variability characteristic of weedy annuals, (2) the fact that important diagnostic characters are present in the seeds, which are of small size and often lacking from collected material, (3) the repetition of similar variations in habit and leaf shape in distinct species and (4) the lack of pubescence characters in most species." The convergence of these factors probably contributed to the confusion that has surrounded *C. littoreum*, this new Californian species, to this day. We are hopeful that future molecular work will clarify some of the confusion in this complex and lead to further elucidation of the relationships among South and North American taxa.

Acknowledgments

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REVIEW

Desert Wisdom/Agaves and Cacti: CO_2, Water, Climate Change. By PARK S. NOBEL. 2010. iUniverse, Inc., New York, NY and Bloomington, IN. 182 pp. ISBN 978-1-4401-9151-0 $16.95, soft cover. ISBN 978-1-4401-9152-7 $6.00, eBook.

Park Nobel is well known to plant biologists interested in plant-environment interactions. From 1979 through 2009, the ISI Web of Science lists 245 peer-reviewed articles he has authored or coauthored and almost all involve the physiology of agaves and cacti. In addition, Nobel is author or editor of four books dealing with agaves and or cacti and is also the author of a unique textbook on environmental plant physiology (Nobel, 2009). So why this new book, *Desert Wisdom*? This book is similar to Nobel's other books in that it draws on a wide range of research that has been conducted on plants in general and agaves and cacti in particular. *Desert Wisdom* differs from its predecessors in that it is written for a broader audience and it takes a position of advocacy for planting agaves and cacti in locations around the globe that are predicted to become hotter and drier. The writing style in this book is less formal than is typical for Nobel but he does not abandon the quantitative perspective that attracts many readers to his work.

Desert Wisdom contains seven chapters. The first chapter stands out from the others in that it focuses on commercial uses of agaves and cacti rather than their environmental physiology. This is interesting reading, particularly if you know little about the historical use of agaves and cacti or their present-day economic importance. Most species of agaves and cacti possess the unusual photosynthetic pathway known as Crassulacean Acid Metabolism (CAM). Chapter two describes CAM's biochemical features and how the CAM pathway improves water conservation. Chapter three explains how well many agaves and cacti tolerate drought and temperature extremes. Chapter four, titled "Issues of Global Climate Change", reviews and defines the problems plants as well as humans will be facing in the future. In the first 16 pages of chapter four, Nobel describes the basis and scope of the problem. He summarizes the main conclusions that can be derived from global change models, particularly with regards to plants. The remainder of chapter four describes how plants and particularly agaves and cacti should be able to adapt to changing climate. Nobel uses a systematic, direct approach to analyze what global change models can tell us. Besides being important to the thesis of this book, I think many readers will find this concise, non alarmist presentation a very useful overview of climate change. In chapter five Nobel explains an Environmental Productivity Index (EPI) that he has developed and which can be used to predict the effect of different environmental factors on net CO_2 uptake. Separate indices for plant response to light, temperature, water availability, nutrient availability, and CO_2 concentration are determined and then these indices are multiplied together. The resulting number or EPI is used to interpret the growth of agaves and cacti in the field. Chapter six is an exploration of plant productivity based on the EPI. The overall ideas in chapters five and six are straight forward to grasp but derivation of the individual indices is not so clear. It took a review of some of the original research citations for this reviewer to understand what must be measured to determine the water availability index, for example. The final chapter summarizes how agaves and cacti should be important players in man's response to climate change. Nobel argues the high productivity of agaves and cacti in hot and dry conditions makes these plants ideal for combating desertification. Agaves and cacti may serve a more direct economic role since they can serve as carbon sinks and provide carbon credits or the related carbon offsets. Agaves and cacti could also be utilized as fodder for livestock or as stocks for biofuels.

Nobel adds clear definitions in the text to minimize jargon and includes a glossary of important terms. I did find the organization of references into separate topics cumbersome. The separate lists are meant to aid those that want to read further about a specific topic. However, checking citations while reading the text is confusing since many references could fit into more than one grouping. I note *Desert Wisdom* is a bargain, listing for $16.95 for the bound copy and $6.00 for an eBook version (see Nobel's website for information: www.eeb.ucla.edu/nobel). The interesting material, logical arguments, and direct writing style make this book an interesting read. Interested non-scientists as well as scientists with wide ranging backgrounds should enjoy and find something new in *Desert Wisdom*.

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NOTEWORTHY COLLECTION

ARIZONA

PUNICA GRANATUM L. (LYTHRACEAE).—Graham Co., river sand dune of Gila River, N of Deadman Canyon, 32.897717°N, −109.467783°W, riparian, a few local shrubs with red flowers, associated species include *Tamarix ramosissima*, *Hymenoclea monogyra*, *Lappula occidentalis*, *Baccharis salicifolia*, *Mentzelia veatchiana*, *Prosopis velutina*, *Baccharis sarothroides*, *Tripterocalyx wootonii*, *Eriogonum trichopes*, *Allionia incarnata*, *Solanum elaeagnifolium*, *Senecio flaccidus*, *Mentzelia multiflora*, *Calycoseris wrightii*, *Hordeum murinum glaucum*, *Stephanomeria exigua*, *Ipomopsis longiflora*, 5 May 2004, *Wendy Hodgson 17923 and Dixie Damrel* (DES). Pinal Co., near Dudleyville, floodplain east side of San Pedro River, ~350 meters from river channel under large *Salix*, 32.926611°N, −110.733278°W, elev. 649 meters, mixed *Populus fremontii*, *Salix gooddingii*, *Tamarix*, *Prosopis velutina* river terrace community, other associated species include *Chloracantha spinosa*, *Sonchus asper*, *Sisymbrium irio*, *Bromus diandrus*, *Bromus madritensis* ssp. *rubens*, *B. catharticus*, *Clematis drummondii*, *Conyza canadensis*, *Hordeum murinum* ssp. *glaucum*, *Matelea producta*, *Hedosyne ambrosiifolia*, *Nicotiana glauca*, *Silybum marianum*, *Rumex* sp., two plants seen, one 1.5 meters tall, the other 0.5 meters tall, flowering and fruiting. 19 Jun 2008, *Michael Denslow 2587 and Elizabeth Ray* (BOON, ASU).

Previous knowledge. Pomegranate is native to western Asia and has been cultivated since antiquity (Davidson 1999). It is reported as introduced in six states in the southern United States including California and Utah (USDA, NRCS 2009). The plant has not previously been reported outside cultivation from Arizona (Shreve and Wiggins 1964; Kearney and Peebles 1969; Lehr 1978; Anonymous 2009; USDA, NRCS 2009). It was likely first introduced into Arizona as a fruit crop though non-fruiting cultivars are also available. Early settlers planted pomegranates near springs (e.g., Quitobaquito in Organ Pipe Cactus National Monument, Bowers 1980). It is widely cultivated and is a common component of many cultivated landscapes today.

Significance. The specimens cited here are from riparian areas and do not appear to be individuals that are persisting from cultivation. The sites were not within human settlements and showed no signs of anthropogenic disturbance. The plants were found to be flowering and fruiting. These are the first reports of this shrub established outside cultivation in the flora of Arizona. Based on these records this species should be sought elsewhere in riparian habitats in Arizona.

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ERRATUM

In the Note by Malcom and Radke (2008), the incorrect family is provided for *Lilaeopsis schaffneriana* var. *recurva*. The correct family is the Apiaceae (e.g., USDA, NRCS 2010).

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